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18F-labeled 1,4-Dioxa-8-azaspiro[4.5]decane Derivative: Synthesis and Biological Evaluation of a Sigma-1 Receptor Radioligand with Low Lipophilicity as Potent Tumor Imaging Agent

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¹⁸F-labeled 1,4-Dioxa-8-azaspiro[4.5]decane Derivative: Synthesis and Biological Evaluation of a Sigma-1 Receptor Radioligand with Low Lipophilicity as Potent Tumor Imaging Agent

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ABSTRACT

We report the syntheses and evaluation of series of novel piperidine compounds with low lipophilicity receptor ligands. as σ_1 8-(4-(2-Fluoroethoxy)benzyl)-1,4-dioxa-8-azaspiro[4,5]decane (5a) possessed high affinity $(K_i = 5.4 \pm 0.4 \text{ nM})$ for σ_1 receptors and selectivity for σ_2 receptors (30-fold) and the vesicular acetylcholine transporter (1404-fold). $[^{18}F]$ 5a was prepared using a one-pot, two-step labeling procedure in an automated synthesis module, with a radiochemical purity of > 95%, and a specific activity of 25–45 GBq/ μ mol. Cellular association, biodistribution and autoradiography with blocking experiments indicated specific binding of $[^{18}F]$ 5a to σ_1 receptors in vitro and in vivo. Small animal positron emission tomography (PET) imaging using mouse tumor xenograft models demonstrated a high accumulation both in human carcinoma and melanoma. Treatment with haloperidol significantly reduced the accumulation of the radiotracer in tumors. These findings suggest that radiotracer with suitable lipophilicity and appropriate affinity for σ_1 receptors could be used for tumor imaging.

Keywords: Carcinoma, melanoma, sigma-1 receptors, piperidine compounds

INTRODUCTION

The sigma (σ) receptor was originally postulated as a class of opioid receptor subtype.¹ Currently, two subtypes of σ receptors were confirmed, namely σ_1 and σ_2 .^{2,3} The σ_1 receptor is a unique 25.3 kDa protein containing 223 amino acids and two transmembrane regions.⁴⁻⁶ It represents a "receptor chaperone" and has been shown to be involved in the regulation of a variety of cellular functions.⁶⁻⁹ They are linked to CNS diseases, such as depression, drug addiction, Parkinson's disease (PD), Alzheimer's disease (AD), tumors and heart failure.⁸⁻¹⁵ On the other hand, the structure of the σ_2 receptor was not identified so far. However, 21.5 kDa was estimated as its molecular weight.² Of note, progesterone receptor membrane component 1 (PGRMC1) was considered as σ_2 binding site recently.¹⁶

Both of the σ receptors were reported to be highly expressed not only in various brain tumors, like glioma and neuroblastoma, but also in a variety of peripheral neoplasia, like breast cancer, malignant melanoma, colon carcinomas, prostate cancer, renal carcinomas, and small cell lung carcinoma.^{12,13,17-22} Higher level of σ_1 receptor expression in cancer cells was found than that in the corresponding non-cancerous cells.²⁰ Furthermore, the σ_2 receptors are also highly expressed in rapidly proliferative cells than in quiescent cells.^{21,22} Thus, the σ receptors represent valuable targets for tumor imaging.

Currently, N-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-2-(2-[¹⁸F]fluoroethoxy)-5-methylbenzamide ([¹⁸F]ISO-1, Figure 1) is in clinical trials for *in vivo* imaging of σ_2 receptors in tumors.^{23,24} Among the investigated σ_1 receptor radiotracer for PET imaging, 1-(3-methoxy-4-[¹¹C]methoxyphenethyl)-4-(3-phenylpropyl)piperazine ([¹¹C]SA4503) and 1-3-[¹⁸F]fluoropropyl-4-((4-cyanophenoxy)-methyl)piperidine ([¹⁸F]FPS) have been used in human brain study.^{25,26} [¹¹C]SA4503 was also found to have a high tumor uptake in C6 tumor bearing rats with tumor-to-plasma and tumor-to-muscle ratios of 13.4 and 5.0, respectively at 1 h postinjection.²⁷ However, this radiotracer seemed to be not suitable as a diagnostic tool for visualization of non small cell lung cancer (NSCLC) and its brain metastases in cancer patients because of the relative high background uptake in lung and brain.²⁸ [¹⁸F]FPS exhibited high B16 melanoma tumor uptake (11.14% ID/g) with tumor-to-blood ratio of 123.8 at 4 h postinjection, but the high uptake and tumor-to-blood ratio were not a result of the σ_1 receptor binding which restricted its application as tumor imaging.²⁹ Until now, there are no successful tumor probes for imaging of σ_1 receptors in the clinical trial.

In the past decade, series of spirocyclic piperidine compounds have been reported as σ_1 ligands.³⁰⁻³⁹ receptor Among these ligands, 1'-benzyl-3-methoxy-3*H*-spiro(2-benzofuran-1,4'-piperidine) (1a) possessed nanomolar affinity for σ_1 receptors and high selectivity against the σ_2 receptors and more than 60 other receptors, transporters and ion channels. ³⁰⁻³² Introduction of the fluorine atom into the carbon side chain of the spirocyclic benzofuran system with two-carbon length led to the discovery of $[^{18}F]$ fluspidine with the potential for neuroimaging and quantitation of σ_1 receptors *in vivo* (Figure 1).³³ In our previous work, we made structural modifications on the *N*-benzyl moiety while eliminating the substituent on the spirocyclic benzofuran section (Figure 2). We introduced an iodine at the *para*-position of the benzyl moiety (compound 2) and found that radioligand $[^{125}I]$ exhibited high initial brain uptake and specific binding to σ_1 receptors in vivo.³⁴ Later, we replaced the iodine atom at the *p*-position of the *N*-benzyl moiety with a fluoro-ethoxylated chain (compound 3). The radioligand 1'-(4-(2-[¹⁸F]fluoroethoxy)benzyl)-3*H*-spiro(2-benzofuran-1,4'-piperidine) ([¹⁸F]**3**) was found to possess subnanomolar affinity and excellent selectivity towards σ_2 receptors and the vesicular acetylcholine transporter (VAChT).³⁵ It also exhibited high initial brain uptake and vivo.³⁵ specific binding to receptors in Moreover, σ_1 1'-benzyl-3-methoxy-3,4-dihydrospiro(2-benzopyran-1,4'-piperidine) (1b) possessed high

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affinity for σ_1 receptors and high subtype selectivity. ³⁰ Replacement of the benzene moiety of spirocyclic compound **1a** and **1b** by pyrazole (**4a**, **4b**) or thiophene residue (**4c**) preserved the affinity and selectivity.³⁶⁻³⁹

In this study, we used $[^{18}F]$ **3** as a lead compound to develop a novel radiotracer for imaging of σ_1 receptors in the tumor. Our design concept is shown in Figure 2. First, we eliminated the benzene ring of the spirocyclic benzofuran moiety and introduced a cyclic acetalic function (5a-5e). The resulting 1,4-dioxa-8-azaspiro[4.5]decane residue (5a) possessed much lower lipophilicity than the former spirocyclic benzofuran piperidine moiety and was thus expected to exhibit fast background washout. We also replaced the 2-fluoroethoxy group of 5a with halogen (5b–5d) or a methoxy group (5e) to assess better affinity and selectivity. Moreover, replaced the *N*-(2-fluoroethoxy)benzyl with we group N-1-(4-fluorophenyl) propan-1-one (6a) or N-1-(4-fluorophenyl) butan-1-one moiety (6b) to examine effects of distance between the basic nitrogen atom and the benzene ring. Finally, by keeping the N-1-(4-fluorophenyl)propan-1-one or N-1-(4-fluorophenyl)butan-1-one moiety constant, we substituted the 1,4-dioxa-8-azaspiro[4.5]decane moiety with simple piperidine residue without or with different substituents such as methyl, hydroxyl and hydroxymethyl (7a-7h) to verify the importance of the *O*-heterocyclic residue. Herein we report the synthesis and evaluation of these compounds as potent σ_1 receptor ligands and their potential applications for imaging σ_1 receptors in the tumor.

RESULTS

Chemistry. The synthetic routes of compounds **5a–5e**, **6a**, **6b** and **7a–7h** are shown in Scheme 1 and Scheme 2, respectively. Compound **8** was obtained from the reaction of *p*-cresol and 1-bromo-2-fluoroethane followed by radical bromination with a total yield of 39% according to the reported method in the literature.³⁵ *N*-alkylation of

1,4-dioxa-8-azaspiro[4.5]decane (9) gave compounds **5a–5e**, **6a** and **6b** with yields of 39–87%. Reactions between compounds **10a–10d** with the corresponding bromides provided compounds **7a–7h** with yields of 42–69%.

In Vitro Radioligand Competition Studies. The affinities of the novel ligands 5a–5e, 6a, 6b and 7a–7h for the σ_1 and σ_2 receptors and for the VAChT were determined with radioligand competition assays as reported previously.^{35,40} (+)-[³H]pentazocine, [³H]-1,3-di-*o*-tolyl-guanidine ([³H]DTG) (in the presence of 1 µM dextrallorphan to mask the σ_1 receptors) and (-)-[³H]-vesamicol were used for the σ_1 and σ_2 receptors and VAChT, respectively.^{35,40} The results are provided in Table 1.

In general, the novel compounds **5a–5e**, **6a** and **6b** with 1,4-dioxa-8-azaspiro[4.5]decane moiety and compounds 7a-7h with simple piperidine moiety still preferred to bind to σ_1 receptors. *H*-spiro(2-benzofuran-1,4'-piperidine) Replacement of moiety with 1,4-dioxa-8-azaspiro[4.5] decane moiety slightly decreased the affinity and selectivity (3 vs **5a**). But this series of compounds (**5a**–**5e**, **6a** and **6b**) still displayed nanomolar affinity for σ_1 receptors with K_i values of 3.3–11.2 nM and high subtype selectivity towards σ_2 receptors $(K_i(\sigma_2)/K_i(\sigma_1) = 16-71)$. Similarly to the findings for the previously reported spirocyclic piperidine derivatives, a small group at the *para*-position of the benzyl group with F, Br, I, OCH₃, and OCH₂CH₂F, still maintained the high affinity and selectivity for the σ_1 receptors (5a-5e).^{34,35} Replacement of 1,4-dioxa-8-azaspiro[4.5] decane moiety with 4-methylpiperidine moiety also preserved nanomolar affinity and subtype selectivity (6b vs 7a). The piperidine group decreased the σ_1 receptor affinity slightly (**6b** vs 7c). However, less lipophilic groups such as OH, CH₂OH at the *para*-position of the piperidine group decreased the affinity and selectivity considerably (6b vs 7e, 7g). If the number of carbon atoms between the carbonyl group attached to 4-fluorophenyl and piperidine moiety was increased from 3 to 4, increased

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affinity both for σ_1 and σ_2 receptors was observed (7a vs 7b, 7c vs 7d, 7e vs 7f, 7g vs 7h), suggesting that the carbon linker length affected the affinity and selectivity of the compounds for σ_1 receptors.

Considering the high affinity and subtype selectivity of compound **5a** for σ_1 receptors as well as convenient radiosynthesis of the corresponding [¹⁸F]**5a**, compound **5a** was further investigated for its affinity for VAChT. Compound **5a** displayed extremely low affinity for VAChT ($K_i = 7.58 \pm 0.98 \mu$ M) and was thus characterized by a higher selectivity for σ_1 receptors (K_i (VAChT)/ K_i (σ_1) = 1404) compared to compound **5** (K_i (VAChT)/ K_i (σ_1) = 799).³⁵

Radiochemistry. The synthesis of [¹⁸F]**5a** is presented in Scheme 3. Reductive amination of compound **9** in the presence of NaBH(OAc)₃ with 4-hydroxybenzaldehyde led to precursor **13** with a yield of 46%. The formation of radiotracer [¹⁸F]**5a** was achieved through a one-pot, two-step radiolabeling procedure, which was developed for the radiosynthesis of ¹⁸F-radiolabeled COX-2 inhibitors.⁴² In the first step, 2-[¹⁸F]fluoroethyl-1-tosylate was prepared from ethylene 1,2-ditosylate by nucleophilic fluorination with [¹⁸F]fluoride. Then precursor **13** in DMF was added to the reaction vial. ¹⁸F-fluroethylation of compound **13** provided the desired radiotracer. After purification via semi-preparative high performance liquid chromatography (HPLC), [¹⁸F]**5a** was obtained in about 15% radiochemical yield (n = 12, decay-corrected) with a radiochemical purity (RCP) of > 95% and a specific activity (A_S) of 25–45 GBq/µmol. The total synthesis time was 100 min.

Evaluation of the Radiolabeled Compound.

Lipophilicity. The apparent distribution coefficient of the ¹⁸F-labeled radiotracer was determined using a shake-flask method as previously reported.^{35,40} The log $D_{7.4}$ value of [¹⁸F]**5a** was 0.81 ± 0.13, indicating a low lipophilicity of this series of σ_1 receptor ligands

with 1,4-dioxa-8-azaspiro[4.5]decane moiety.

In Vitro Stability. The *in vitro* stability of $[^{18}F]$ **5a** in ethanol, saline, or human plasma was evaluated by measuring the RCP at different time points. After 2 h of incubation with ethanol, saline, or human plasma, the RCP of $[^{18}F]$ **5a** was more than 92% as shown in the Supporting Information.

Cell Uptake Experiments. Four different human tumor cell lines, comprising carcinoma (PC3, DU145, MCF7) and melanoma (A375), which show different level of expression of σ_1 or/and σ_2 receptors were used to study the cellular association of compound [¹⁸F]**5a** in vitro (Figure 3). An increase in the radiotracer association was observed with longer incubation time. Especially in DU145 cells with high density of σ_1 receptors,¹⁹ substantial cellular association of $[^{18}F]$ **5a** (18.55% ID/mg protein) was found at 120 min. The cellular association of [¹⁸F]5a was about 9.73% and 8.08% ID/mg protein in A375 and PC3 cell lines, respectively. The lowest cellular association (5.48% ID/mg protein at 120 min) was observed in MCF7 cell lines, which was in good agreement with their low expression of σ_1 receptors.¹⁷ Blocking studies were performed with haloperidol, SA4503 and fluspidine as blocking agents. After incubation with 10 μ M of haloperidol, SA4503 or fluspidine, the cellular association was significantly reduced in the above four tumor cell lines. Pre-incubation with haloperidol and SA4503 led to marked reduction of 85% and 88% in DU145 cell lines (2.85 and 2.26% vs 18.55% ID/mg protein) at 120 min. Pre-incubation with haloperidol, SA4503 and fluspidine also resulted in a significant blocking of cellular association in A375 and PC3 cell lines (2.81–4.99% ID/mg protein). Moreover, a further reduction was observed when pre-incubated with haloperidol and SA4503 (3.28 and 2.85% ID/mg protein, respectively) in MCF7 cell lines. These data demonstrated specific binding of $[^{18}F]$ 5a to σ_1 receptors in

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carcinoma (PC3, DU145, MCF7) and melanoma (A375) cell lines.

Biodistribution Studies in Rats. To evaluate the kinetics of [¹⁸F]**5***a*, biodistribution studies were performed in male Wistar rats without (control) and with simultaneous injection of haloperidol (1 mg/kg body weight) as blocking agent. The results are summarized in Figure 4 and in the Supporting Information. The standardized uptake value (SUV) and percentage of injected dose (%ID) are applied to evaluate the accumulation of radiotracer in rat organs and tissues.

In controls, [¹⁸F]**5a** displayed high initial brain uptake (3.67 %ID and SUV, 2.89) with high brain-to-blood ratio of 7.81 at 5 min postinjection. Among peripheral organs, the pancreas exhibited the highest uptake (SUV, 6.33 ± 1.15), followed by the liver, adrenals, kidneys, spleen, and lung, which is in accordance with the σ_1 receptor densities in these organs. In addition, the above organs with high expression of σ_1 receptors displayed a relatively fast clearance rate of 31–63% after 60 min.

To detect the specific binding of [¹⁸F]**5a** to σ receptors *in vivo*, the effects of simultaneous injection of haloperidol on the biodistribution of the radiotracer in various organs in male Wistar rats were examined. A remarkable reduction of the radiotracer accumulation (55%) was observed in the brain at 5 min postinjection. Moreover, radiotracer accumulation in the pancreas, lungs and spleen, which are known to possess high expression of σ_1 receptors, was significantly reduced by 48%, 51% and 52%, respectively. At 60 min, the uptake in brain, pancreas, spleen and liver was decreased by 25–64%. These results indicated the specific binding of [¹⁸F]**5a** to σ receptors *in vivo*.

Imaging of Rat Brains. To further confirm the specific binding of $[^{18}F]$ **5a** to σ receptors *in vivo*, micro-PET/MRI and autoradiography studies were performed in adult male Wistar rats.

The binding specificity was evaluated in a blocking study with simultaneous injection of haloperidol (1 mg/kg body weight). Representative brain images (projections and sections) of the radiotracer at 5 and 60 min postinjection and muscle sections of identical animals for comparison are presented in Figure 5. [¹⁸F]**5a** crossed the blood-brain barrier (BBB) rapidly and showed high accumulation in the brain regions such as cortex, cerebellum, and thalamus known to express high density of σ_1 receptors at 5 min postinjection. When animals were simultaneously injected with haloperidol, accumulation of the radiotracer decreased in the above brain regions, consistent with the results of the biodistribution studies.

P-glycoprotein (P-gp) is highly expressed in the blood-brain barrier and many tumors. To test whether $[^{18}F]$ **5a** is a substrate for P-gp *in vivo*, dynamic PET brain imaging with cyclosporine A, an inhibitor of P-gp, was carried out in Wistar rats (n = 2). Either saline or cyclosporine A (5 mg/kg body weight) was injected 10 min prior to the radiotracer injection. The results are summarized in Figure 6. Administration of cyclosporine A did not change the bioavailability of the radiotracer as represented by the area under the curve values (AUCs). The small difference between AUCs in control and that in blocked animals was not significant, suggesting that $[^{18}F]$ **5a** is not a substrate for P-gp.

Small Animal PET Imaging in Mouse Tumor Xenograft Models. To further evaluate the potential applications of [¹⁸F]**5a** for imaging of σ_1 receptors in tumors, small animal dynamic PET studies were performed in four different xenograft models. The results are presented in Figure 7. Among the investigated models, the highest accumulation of the radiotracer was observed in the PC3 tumors (human prostate cancer, n = 3), followed by A431 (human squamous cell carcinoma, n = 5), A375 (human melanoma, n = 2), and DU145 (human prostate cancer, n = 4) tumors. Administration of haloperidol (1 mg/kg body weight) significantly reduced the accumulation of the radiotracer in PC3 (n = 2) or A431 (n = 5)

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tumors (p < 0.05), indicating specific binding of [¹⁸F]**5a** to σ receptors in these tumors *in vivo*.

Representative PET imaging with [¹⁸F]**5a** in NMRI *nu/nu* mice bearing four different tumors is presented in Figure 8. Perfusion background and radiotracer accumulation in an A431 tumor bearing NMRI *nu/nu* mouse are depicted in images A (midframe time 3 min postinjection) and B (midframe time 50 min postinjection), respectively. The signal intensity in image A subtracted that in image B resulted in difference image C clearly illustrating tumor accumulation with high tumor-to-background ratio. Substantial tumor accumulation of [¹⁸F]**5a** and high tumor-to-background ratio were also observed in (D) A375, (E) PC3, and (F) DU145 tumor bearing NMRI *nu/nu* mice. Additionally, PET imaging in mice showed substantial accumulation of radiotracer in intestine and bladder indicating the major elimination routes (Figure 8). A partial contribution of radiotracer accumulation in pancreas to the overall radiotracer accumulation in the abdominal region should be considered and would be consistent with biodistribution data obtained in rats. However, this cannot be differentiated in detail using the imaging approach used in this study.

In Vivo Metabolic Stability. Metabolic stability of [¹⁸F]**5a** was investigated in arterial blood plasma, urine, and brain samples from Wistar rats. The HPLC chromatograms of the radioactive compounds in arterial blood plasma samples at 0, 5, and 60 min postinjection and in a urine sample at 60 min postinjection are presented in the Supporting Information. Metabolite fraction of [¹⁸F]**5a** in arterial blood plasma is shown in the Supporting Information. The HPLC chromatograms of the radiotracer in brain and blood plasma samples at 5 min postinjection without and with haloperidol blocking is provided in the Supporting Information. In arterial blood plasma samples, three less lipophilic metabolites, M1, M2, and M3 were observed at retention times of 4.3, 10.3 and 10.9 min, respectively. At 60 min, the

polar metabolite M1 was found to be the main metabolite. The biological half-life of [¹⁸F]**5a** in arterial blood plasma was 4.1 min. In control brain samples, the intact parent tracer accounted for about 95% of the total activity, and only very small amounts of the metabolite M1 was observed. Treatment of haloperidol resulted in an increment of metabolite M1 in the brain.

DISCUSSION

Experimental and clinical evidence suggest that both σ_1 and σ_2 receptors could play a significant role in cancer biology. Until now, [¹⁸F]ISO-1 is the most successful σ_2 tumor probe.^{23,24} However, there is no successful tumor probe for imaging of σ_1 receptors in clinical trial. The high background uptake of [¹¹C]SA4503 and the non-specific binding of [¹⁸F]FPS in B16 tumor restricted their applications in tumor detection.^{12,28,29} If a σ_1 receptor probe is more hydrophilic, its background uptake is expected to be lower and thus advantageously for tumor imaging. Based on these prerequisites, our aim was to develop a tumor radiotracer with suitable lipophilicity and appropriate affinity for σ_1 receptors.

In our previous work, we found that compound **3** possessed subnanomolar affinity and high selectivity for σ_1 receptors. Moreover, [¹⁸F]**3** showed very high brain uptake and specific binding to σ_1 receptors *in vivo*.³⁵ For this series of spirocyclic piperidine derivatives, Wünsch considered the benzene ring of the *O*-heterocycle as the "primary hydrophobic region" and the phenyl group of the *N*-substituent as the "secondary hydrophobic region" of the σ_1 ligands by Glennon's pharmacophore model.³⁹ Recently, several σ_1 receptor residues have been identified to play a central role in binding of different series of structurally unrelated compounds.⁴³ In order to design compounds with lower liphophilicity and nanomolar affinity, we replaced the spirocyclic piperidine moiety in compound **3** with a more hydrophilic group 1,4-dioxa-8-azaspiro[4.5]decane and simple piperidine. *In vitro* binding assays showed that

5a–5e, **6a** and **6b** with 1,4-dioxa-8-azaspiro[4.5]decane and **7a**, **7b** with simple piperidine still exhibited nanomolar affinity and subtype selectivity for σ_1 receptors. Moreover, compound **5a** also exhibits 1404-fold less affinity for VAChT. That is to say, the lack of the benzene moiety connected to the spirocyclic piperidine residue could preserve the nanomolar affinity for σ_1 receptors while it could decrease the affinity for VAChT. Some other less lipophilic and smaller moieties, like methyl may serve as "primary hydrophobic region" in the piperidine ligands. And thus the "primary hydrophobic region" in the Glennon's pharmacophore model appears more flexible.

Considering nanomolar affinity and comparable selectivity to that of [¹¹C]SA4503 as well as the convenient synthesis of the corresponding radiotracer, [¹⁸F]**5a** was prepared to detect the kinetic profiles *in vivo*. For brain imaging probes, moderate lipophilicity (log D = 1-3) of the tracers is considered to be favorable for penetrating the BBB.⁴⁴ But for tumor imaging agent, suitable lipophilicity needs to be optimized in order to find the potential radiotracer with high tumor uptake and low background accumulation. Consistent with our prediction, the lipophilicity of [¹⁸F]**5a** (log $D_{7.4} = 0.81$) is much lower than that of [¹¹C]SA4503 (log $D_{7.4}$ = 2.52),⁴⁵ [¹⁸F]FPS (log $D_{7.5} = 2.80$),⁴⁶ [¹⁸F]fluspidine (log $D_{7.2} = 2.57$),³³ and [¹⁸F]**3** (log $D_{7.4}$ = 2.41).³⁵ Moreover, [¹⁸F]**5a** displayed high *in vitro* stability in ethanol, saline and human plasma at room temperature (r. t.) for 2 h. These *in vitro* properties encouraged us to perform the *in vivo* evaluation of [¹⁸F]**5a** as tumor imaging agent.

In biodistribution studies in rats, [¹⁸F]**5a** (SUV, 2.89) showed higher brain uptake than that of [¹¹C]SA4503 (SUV, 1.50 in cortex and SUV, 1.66 in cerebellum at 60 min postinjection, SUV, 1.55 in cortex and SUV, 1.21 in cerebellum at 90 min postinjection) in rats.⁴⁷⁻⁴⁹ In addition, this radiotracer exhibited low initial blood uptake and thus high brain-to-blood ratio. Simultaneous administration of haloperidol resulted in a significantly decreased accumulation in organs and tissues with high expression of σ_1 receptors, including brain, liver, lung, kidney, and pancreas, indicating specific binding of $[^{18}F]$ **5a** to σ receptors *in vivo*.

In autoradiography studies in rats, high accumulation of the radiotracer was observed in brain areas with high expression of σ_1 receptors, such as cortex, cerebellum, and thalamus at 5 min postinjection. Treatment with haloperidol also reduced the accumulation of [¹⁸F]**5a** in these regions. The results of rat brain PET images in control and blocking animals were in good agreement with those of the autoradiography. Cerebellum, thalamus, and cortex containing high density of σ_1 receptors showed high accumulation. Blocking with haloperidol and SA4503 significantly decreased the uptake, especially in these regions. Considering the low affinity of **5a** for VAChT and the high selectivity of SA4503 towards 36 other receptors, ion channels and components of second messenger systems,⁵⁰ we conclude that the results of the blocking study suggest specific binding of [¹⁸F]**5a** to σ_1 receptors in the rat brain *in vivo*.

It is well known that P-gp is highly expressed at the blood-brain barrier and in tumors. The identification of a radiotracer as a substrate of P-gp is very important in the development of brain or tumor imaging agent.⁵¹ Co-injection of cyclosporine A with [¹⁸F]**5a** did not increase the brain uptake significantly (p > 0.05) in the PET studies indicating that the radiotracer is not a P-gp substrate. This finding may explain the high initial brain uptake of [¹⁸F]**5a** despite low lipophilicity and encourage us to further evaluate this radiotracer as tumor imaging agent.

In order to confirm the specific binding [¹⁸F]**5a** to σ_1 receptors in tumor cell lines, the cellular association of [¹⁸F]**5a** was measured in four kinds of cell lines with different levels of σ_1 receptor expression. Among the four cell lines, DU145 cells were reported to possess high expression of σ_1 and σ_2 receptors with B_{max} values of 1,800 and 1,930 fmol/mg protein, respectively.¹⁹ High σ_1 receptor expression in PC3 cell line investigated with real time PCR was also reported. A375 cells exhibited relatively low expression of σ_1 receptors but high expression of σ_2 receptors (with B_{max} values 34 and 3,403 fmol/mg protein toward σ_1 and σ_2

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receptors, respectively).^{17,20} Even lower σ_1 receptor expression was reported in MCF7 cells.^{17,20} Consistent with the expression levels of σ_1 receptors in these cells, highest cellular association of [¹⁸F]**5a** was observed in DU145 cells, followed by A375, PC3 and MCF7 cells. Treatment with SA4503 (σ_1 agonist with nanomolar affinity and high selectivity),^{41,48,52} haloperidol (a potent σ_1 and σ_2 antagonist)^{53,54} or fluspidine (a potent σ_1 probe)³³ reduced the cellular association of [¹⁸F]**5a** significantly. These results demonstrated the specific binding of [¹⁸F]**5a** to σ_1 receptors in these cell lines *in vitro*.

To further determine the specific binding in tumors *in vivo*, small animal dynamic PET studies were investigated in four different xenograft models in mice. Significant radiotracer accumulation in the tumors of the radiotracer was observed in all models. Different from what was found in the cellular association studies, the highest tumor uptake was obtained in the PC3 tumor, followed by in the A431, A375 and DU145 tumor. Such discrepancy may be, in part, due to differences between the used carcinomas and melanoma in tumor growth rate, vascularization, perfusion and cellular microenvironment. However, the uptake in these tumor bearing mice was higher than those of [11 C]SA4503 (SUV, 0.65 ± 0.15) and [18 F]FE-SA5845 (SUV, 0.92 ± 0.39) in the C6 tumor.²⁷ Treatment with haloperidol significantly reduced the accumulation in the PC3 and A431 tumors, which is similar to the uptake of [11 C]SA4503 in the C6 tumor (SUV, 0.43 ± 0.15).²⁷ These data indicate specific binding of [18 F]**5a** to σ_1 receptors in the tumors *in vivo*. Moreover, PET revealed considerable tumor uptake and a significant blocking effect of haloperidol in A431 tumor bearing NMRI *nu/nu* mice. These results further demonstrate the promising features of [18 F]**5a** as tumor imaging agent.

CONCLUSION

Series of compounds with more hydrophilic group 1,4-dioxa-8-azaspiro[4.5]decane and

simple piperidine moieties were synthesized and found to possess nanomolar affinity, subtype selectivity for σ_1 receptors and high selectivity for VAChT. The more flexible "primary hydrophobic region" in the Glennon's pharmacophore model provides the chance to develop σ_1 receptor radiotracers with low lipophilicity for tumor imaging. The novel radiotracer [¹⁸F]**5a** not only possessed the nanomolar affinity and selectivity for σ_1 receptors, but also showed specific binding to σ_1 receptors in the normal organs known to express high level of σ_1 receptors, tumor cell lines, and tumors *in vivo*. The clear visualization of the tumor in PET images with this radiotracer indicates a progress in the development of σ_1 receptor radiotracers are planned.

EXPERIMENTAL SECTION

General Methods and Materials. All the chemicals or reagents were purchased from commercial suppliers and used without further purification.¹H NMR, ¹³C NMR, and ¹⁹F NMR spectra were recorded on a Varian Inova-400 spectrometer at 400, 101, and 376 MHz, respectively. Chemical shifts are reported in ppm with tetramethylsilane (¹H, ¹³C) and trichlorofluoromethane (¹⁹F) as internal standards, respectively and coupling constants (*J*) are reported in Hertz (Hz). MS spectra were obtained by Quattro micro API ESI/MS (Waters, USA) and Quattro micro WATERS Xevo TQ-S. High resolution mass spectrometry (HRMS) was performed on a JEOL NMS-SX102 spectrometer (JEOL). HPLC analyses were performed using a Luna C18 column (250 × 4 mm, 5 µm) (Phenomenex) using an isocratic eluent of acetonitrile/Na₂HPO₄ (0.05 M) = 50/50 by a gradient pump L2500 (Merck, Hitachi) and Agela Venusil MP C18 (5 µm, 4.6 × 250 mm) using CH₃CN/NH₄OAc (0.01 M) by Shimadzu SCL-20 AVPHPLC system with a flow rate of 1 mL/min. The products were

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monitored by a UV detector L4500 (Merck, Hitachi) and SPD-M20AUV-VIS detector at 254 nm and by γ -detection with a scintillation detector Gabi (Raytest). The purities of the final products were determined by the HPLC method, all the final compounds were higher than 95% and the purities are shown in the Supporting Information. No-carrier-added aqueous [¹⁸F]fluoride was produced using a CYCLONE 18/9 cyclotron (IBA) by irradiation of [¹⁸O]H₂O via the ¹⁸O(*p*,*n*)¹⁸F nuclear reaction. Synthesis of [¹⁸F]**5a** was performed in an automated nucleophilic synthesizer TracerLAB_{FXN} (GE). Semi-preparative purification was performed with a Nucleosil-100 C18 column (250 × 21 mm, 7 µm) (Macherey-Nagel) using an isocratic eluent of acetonitrile/Na₂HPO₄ (0.05 M) = 60/40 by a S1122 HPLC pump (Sykam) with a flow rate of 5 mL/min. The product was monitored by a K-2001 filer photometer (Knauer) at 254 nm and by a γ -detector integrated in the synthesis module.

Animal experiments were carried out according to the guidelines of the German Regulations for Animal Welfare. The protocol was approved by the local Ethical Committee for Animal Experiments.

Chemistry.

General Procedure for the Syntheses of 5a–5e, 6a, 6b and 7a–7h. All of these products were synthesized under the similar conditions. 1.0 Equiv of the corresponding bromide compound, 1.2 equiv of 1,4-dioxa-8-azaspiro[4.5]decane or corresponding piperidine, K₂CO₃, and NaI in catalytic amounts were added into the CH₃CN, the mixture was refluxed for 4 h. After cooling and filtration, the solvent was removed under reduced pressure. Finally, the crude product was purified by column chromatography (silica gel, petroleum ether:ethyl acetate = 1:2 or 1:1 (ν/ν)) to afford the final products.

8-(4-(2-Fluoroethoxy)benzyl)-1,4-dioxa-8-azaspiro[4.5]decane (5a). Compound **8** (306 mg, 1.31 mmol), compound **9** (225 mg, 1.57 mmol), K₂CO₃ (217 mg, 1.57 mmol), and NaI in

catalytic amounts were added into CH₃CN (30 mL) and afforded **5a** (208 mg, 54%) as light-yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 7.20 (d, J = 8.5 Hz, 2H), 6.84 (d, J = 8.5 Hz, 2H), 4.72 (dt, J = 47.4, 4.2 Hz, 2H), 4.16 (dt, J = 27.8, 4.2 Hz, 2H), 3.91 (s, 4H), 3.43 (s, 2H), 2.47 (s, 4H), 1.70 (t, J = 5.6 Hz, 4H). ¹³C NMR (101 MHz, CDCl₃) δ 157.7, 131.5, 130.5, 114.5, 107.5, 82.2, 67.3, 64.4, 62.2, 51.4, 35.0. ¹⁹F NMR (376 MHz, CDCl₃) δ –224.3. MS (ESI+) m/z: 295.92 [M + H]⁺. HRMS (EI) m/z: calcd for C₁₆H₂₂FNO₃ [M + H]⁺ 296.1662, found 296.1653.

8-(4-Fluorobenzyl)-1,4-dioxa-8-azaspiro[4.5]decane

(5b).

1-(Bromomethyl)-4-fluorobenzene (436 mg, 2.31 mmol), compound **9** (395 mg, 2.76 mmol), K₂CO₃ (319 mg, 2.31 mmol), and NaI in catalytic amounts were added into CH₃CN (25 mL) and afforded **5b** (344 mg, 59%) as yellow oil. ¹H NMR(400 MHz, CDCl₃) δ 7.33–7.22 (m, 2H), 6.93–7.00 (m, 2H), 3.92 (s, 4H), 3.46 (s, 2H), 2.49 (s, 4H), 1.71 (t, *J* = 5.4 Hz, 4H). ¹³C NMR (101 MHz, CDCl₃) δ 161.9, 134.3, 130.4, 114.9, 107.2, 64.2, 61.8, 51.2, 34.8. ¹⁹F NMR (376 MHz, CDCl₃) δ –224.4. MS (ESI+) *m/z*: 251.95 [M + H]⁺. HRMS (EI) *m/z*: calcd for C₁₄H₁₈FNO₂ [M + H]⁺ 252.1400, found 252.1409.

8-(4-Iodobenzyl)-1,4-dioxa-8-azaspiro[4.5]decane (5c). 1-(Bromomethyl)-4-iodobenzene (520 mg, 1.76 mmol), compound 9 (301 mg, 2.10 mmol), K₂CO₃ (150 mg, 2.10 mmol), and NaI in catalytic amounts were added into CH₃CN (35 mL) and afforded 5c (392 mg, 62%) as light-yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 7.60 (d, *J* = 8.1 Hz, 2H), 7.06 (d, *J* = 8.0 Hz, 2H), 3.91 (s, 4H), 3.43 (s, 2H), 2.48 (s, 4H), 1.71 (t, *J* = 5.5 Hz, 4H). ¹³C NMR(101 MHz, CDCl₃) δ 138.4, 137.2, 130.9, 107.1, 92.3, 64.2, 62.0, 51.2, 34.8. MS (ESI+) *m/z*: 359.77 [M + H]⁺. HRMS (EI)*m/z*: calcd for C₁₄H₁₈INO₂ [M + H]⁺360.0461, found 360.0455.

8-(4-Bromobenzyl)-1,4-dioxa-8-azaspiro[4.5]decane

1-Bromo-4-(bromomethyl)benzene (400 mg, 1.60 mmol), compound **9** (275 mg, 1.92 mmol), K₂CO₃ (265 mg, 1.92 mmol), and NaI in catalytic amounts were added into CH₃CN (25 mL)

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and afforded **5d** (437 mg, 87%) as light-yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 7.37 (d, *J* = 8.3 Hz, 2H), 7.15 (d, *J* = 8.2 Hz, 2H), 3.88 (s, 4H), 3.41 (s, 2H), 2.45 (s, 4H), 1.68 (t, *J* = 5.6 Hz, 4H). ¹³C NMR(101 MHz, CDCl₃) δ 132.8, 126.5, 125.9, 116.0, 102.4, 59.4, 57.1, 46.4, 30.0. MS (ESI+) *m/z*: 314.08 [M + H, ⁸¹Br], 312.02 [M + H, ⁷⁹Br]⁺. HRMS (EI) *m/z*: calcd for C₁₄H₁₈BrNO₂ [M + H]⁺312.0599, found 312.0614.

8-(4-Methoxybenzyl)-1,4-dioxa-8-azaspiro[4.5]decane

(5e).

1-(Bromomethyl)-4-methoxybenzene (760 mg, 3.78 mmol), compound **9** (649 mg, 4.54 mmol), K₂CO₃ (626 mg, 4.54 mmol), and NaI in catalytic amounts were added into CH₃CN (45 mL) and afforded **5e** (708 mg, 71%) as yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 7.20 (d, J = 8.6 Hz, 2H), 6.82 (d, J = 8.6 Hz, 2H), 3.91 (s, 4H), 3.77 (s, 3H), 3.44 (s, 2H), 2.48 (s, 4H), 1.71 (t, J = 5.6 Hz, 4H). ¹³C NMR(101 MHz, CDCl₃) δ 158.7, 130.2, 113.5, 110.0, 107.3, 64.2, 62.0, 55.2, 51.1, 34.7. MS (ESI+) *m/z*: 264.09 [M + H]⁺. HRMS (EI) *m/z*: calcd for C₁₅H₂₁NO₃ [M + H]⁺264.1600, found 264.1607.

1-(4-Fluorophenyl)-3-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)propan-1-one (6a).

3-Bromo-1-(4-fluorophenyl)propan-1-one (620 mg, 2.68 mmol), compound **9** (460 mg, 3.22 mmol), K₂CO₃ (444 mg, 3.22 mmol), and NaI in catalytic amounts were added into CH₃CN (25 mL) and afforded **6a** (303 mg, 39%) as light-yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 7.96–7.86 (m, 2H), 7.11–7.01 (m, 2H), 3.88 (s, 4H), 3.09 (t, J = 7.4 Hz, 2H), 2.79 (t, J = 7.4 Hz, 2H), 2.53 (t, J = 5.0 Hz, 4H), 1.68 (t, J = 5.6 Hz, 4H). ¹³C NMR(101 MHz, CDCl₃) δ 197.6, 165.7, 133.4, 130.6, 115.6, 107.0, 64.2, 52.7, 51.4, 36.5, 34.8. ¹⁹F NMR (376 MHz, CDCl₃) δ –106.4. MS (ESI+) *m/z*: 293.95 [M + H]⁺. HRMS (EI)*m/z*:calcd for C₁₆H₂₀FNO₃ [M + H]⁺ 294.1505, found 294.1510.

1-(4-Fluorophenyl)-4-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)butan-1-one (6b).

4-Bromo-1-(4-fluorophenyl)butan-1-one (300 mg, 1.22 mmol), compound **9** (210 mg, 1.47 mmol), K₂CO₃ (202 mg, 1.47 mmol), and NaI in catalytic amounts were added into CH₃CN

(35 mL) and afforded **6b** (168 mg, 45%) as colorless solid. ¹H NMR (400 MHz, CDCl₃) δ 7.98–7.90 (m 2H), 7.12–7.00 (m, 2H), 3.87 (s, 4H), 2.92 (t, J = 7.1 Hz, 2H), 2.49 (s, 4H), 2.40 (t, J = 7.1 Hz, 2H), 1.95–1.84 (m, 2H), 1.65 (t, J = 5.6 Hz, 4H). ¹³C NMR(101 MHz, CDCl₃) δ 193.6, 160.8, 128.8, 125.9, 110.8, 102.3, 59.4, 52.4, 46.4, 31.4, 29.8, 17.1. ¹⁹F NMR (376 MHz, CDCl₃) δ –106.2. MS (ESI+) *m/z*: 308.21 [M + H]⁺. HRMS (EI) *m/z*: calcd for C₁₇H₂₂FNO₃ [M + H]⁺ 308.1662, found 308.1654.

1-(4-Fluorophenyl)-4-(4-methylpiperidin-1-yl)butan-1-one (7a). Compound **11**, 4-bromo-1-(4-fluorophenyl)butan-1-one (160 mg, 0.65 mmol), 4-methylpiperidine (83 mg, 0.84 mmol), K₂CO₃ (116 mg, 0.84 mmol) and NaI in catalytic amounts were added into CH₃CN (25 mL) afforded **7a** (118 mg, 69%).¹H NMR (400 MHz, CDCl₃) δ 8.04–7.93 (m, 2H), 7.11 (t, *J* = 8.5 Hz, 2H), 3.05–2.98 (m, 2H), 2.54 (t, *J* = 7.4 Hz, 4H), 2.11 (t, *J* = 11.1 Hz, 2H), 2.07–1.94 (m, 2H), 1.64 (d, *J* = 11.0 Hz, 2H), 1.46–1.26 (m, 3H), 0.92 (d, *J* = 5.7 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 198.1, 165.6, 133.3, 130.6, 115.6, 57.6, 53.5, 36.1, 33.2, 30.3, 21.5, 20.9. ¹⁹F NMR (376 MHz, CDCl₃) δ –106.52. MS (ESI⁺): *m/z* 264.06 [M+H]⁺. HRMS (EI)*m/z*:calcd for C₁₆H₂₃FNO [M + H]⁺ 264.1764, found 264.1773.

1-(4-Fluorophenyl)-5-(4-methylpiperidin-1-yl)pentan-1-one (7b). Compound **12**, 5-bromo-1-(4-fluorophenyl)pentan-1-one (300 mg, 1.15 mmol), 4-methylpiperidine (146 mg, 1.47 mmol), K₂CO₃ (202 mg, 1.47 mmol) and catalytic amount NaI were added into CH₃CN (40 mL) and afforded 7b (278 mg, 63%). ¹H NMR (400 MHz, CDCl₃) δ 7.99–7.94 (m, 2H), 7.10 (t, *J* = 8.4 Hz, 2H), 2.94 (t, *J* = 7.3 Hz, 2H), 2.88 (d, *J* = 11.5 Hz, 2H), 2.35 (t, *J* = 11.0 Hz, 2H), 1.90 (t, *J* = 11.2 Hz, 2H), 1.78–1.68 (m, 2H), 1.63–1.54 (m, 4H), 1.39–1.18 (m, 3H), 0.89 (d, *J* = 6.2 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 198.5, 165.6, 133.3, 130.6, 115.5, 58.6, 54.0, 38.3, 34.2, 30.7, 26.5, 22.4, 21.8. ¹⁹F NMR (376 MHz, CDCl₃) δ –105.72. MS (ESI⁺): *m/z* 278.21 [M+H]⁺. HRMS (EI)*m/z*:calcd for C₁₇H₂₅FNO [M + H]⁺ 278.1920, found 278.1928.

1-(4-Fluorophenyl)-4-(piperidin-1-yl)butan-1-one (7c). Compound 11 (426 mg, 1.73 mmol), piperidine (188 mg, 2.21 mmol), K₂CO₃ (305 mg, 2.21 mmol) and NaI in catalytic amounts were added into CH₃CN (40 mL) and afforded 7c (296 mg, 69%). ¹H NMR (400 MHz, CDCl₃) δ 7.96–7.83 (m, 2H), 6.99 (t, J = 8.5 Hz, 2H), 2.86 (t, J = 7.0 Hz, 2H), 2.32 (t, J = 6.9 Hz, 6H), 1.94–1.78 (m, 2H), 1.53–1.41 (m, 4H), 1.37–1.23 (m, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 193.4, 160.7, 128.6, 125.8, 110.7, 53.3, 49.4, 31.4, 20.6, 19.3, 16.4. ¹⁹F NMR (376 MHz, CDCl₃) δ –106.81. MS (ESI⁺): m/z 249.88 [M+H]⁺. HRMS (EI)m/z:calcd for C₁₅H₂₁FNO [M + H]⁺ 250.1607, found 250.1606.

1-(4-Fluorophenyl)-5-(piperidin-1-yl)pentan-1-one (7d). Compound **12** (200 mg, 0.77 mmol), piperidine (140 mg, 0.98 mmol), K₂CO₃ (135 mg, 0.95 mmol) and NaI in catalytic amounts were added into CH₃CN (20 mL) and afforded **7d** (85 mg, 42%). ¹H NMR (400 MHz, CDCl₃) δ 7.98–7.89 (m, 2H), 7.07 (t, *J* = 8.6 Hz, 2H), 2.91 (t, *J* = 7.3 Hz, 2H), 2.38–2.23 (m, 6H), 1.78–1.64 (m, 2H), 1.60–1.48 (m, 6H), 1.40–1.34 (m, *J* = 2.2 Hz, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 193.8, 160.8, 128.6, 125.9, 110.9, 54.3, 49.8, 33.6, 21.7, 21.2, 19.7, 17.7. ¹⁹F NMR (376 MHz, CDCl₃) δ –106.74. MS (ESI⁺): *m/z* 265.05 [M+H]⁺. HRMS (EI)*m/z*:calcd for C₁₆H₂₃FNO [M + H]⁺ 264.1764, found 264.1766.

1-(4-Fluorophenyl)-4-(4-hydroxypiperidin-1-yl)butan-1-one (7e). Compound **11** (384 mg, 1.57 mmol), 4-hydroxypiperidine (190 mg, 1.88 mmol), K₂CO₃ (259 mg, 0.74 mmol) and NaI in catalytic amounts were added into CH₃CN (50 mL) and afforded **7e** (275 mg, 66%). ¹H NMR (400 MHz, CDCl₃) δ 8.04–7.95 (m, 2H), 7.13 (t, *J* = 8.7 Hz, 2H), 3.85 (s, 1H), 3.05 (t, *J* = 6.9 Hz, 2H), 2.99–2.85 (m, 2H), 2.63 (t, *J* = 7.3 Hz, 2H), 2.52 (s, 2H), 2.17–2.01 (m, 4H), 1.76 – 1.56 (m, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 197.9, 165.7, 133.2, 130.7, 115.7, 66.8, 57.2, 50.1, 36.0, 32.9, 20.6. ¹⁹F NMR (376 MHz, CDCl₃) δ –106.21. MS (ESI⁺): *m/z* 266.16 [M+H]⁺.

1-(4-Fluorophenyl)-5-(4-hydroxypiperidin-1-yl)pentan-1-one (7f). Compound 12 (195

mg, 0.75 mmol), 4-hydroxypiperidine (91 mg, 0.90 mmol), K₂CO₃ (124 mg, 0.90 mmol) and NaI in catalytic amounts were added into CH₃CN (25 mL) and afforded **7f** (107 mg, 52%). ¹H NMR (400 MHz, CDCl₃) δ 8.01–7.94 (m, 2H), 7.16–7.07 (m, 2H), 3.80–3.71 (m, 1H), 2.97 (t, J = 7.1 Hz, 2H), 2.89–2.81 (m, 2H), 2.46 (t, J = 7.6 Hz, 2H), 2.33–2.24 (m, 2H), 2.02–1.93 (m, 2H), 1.79–1.57 (m, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 198.5, 165.6, 133.3, 130.61, 115.6, 67.0, 58.0, 50.7, 38.1, 33.7, 26.2, 22.1. ¹⁹F NMR (376 MHz, CDCl₃) δ –106.48. MS (ESI⁺): m/z 280.15 [M+H]⁺.

1-(4-Fluorophenyl)-4-(4-(hydroxymethyl)piperidin-1-yl)butan-1-one (7g). Compound **11** (309 mg, 1.26 mmol), 4-piperidinemethanol (174 mg, 1.51 mmol), K₂CO₃ (208 mg, 1.51 mmol) and NaI in catalytic amounts were added into CH₃CN (35 mL) and afforded **7g** (217 mg, 62%). ¹H NMR (400 MHz, CDCl₃) δ 8.07–7.91 (m, 2H), 7.16–7.04 (m, 2H), 3.47 (d, *J* = 6.4 Hz, 2H), 3.09–2.92 (m, 4H), 2.47 (t, *J* = 7.3 Hz, 2H), 2.11–1.94 (m, 4H), 1.73 (d, *J* = 13.0 Hz, 2H), 1.59–1.46 (m, 1H), 1.37–1.21 (m, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 198.3, 165.6, 133.4, 130.6, 115.6, 67.6, 57.8, 53.3, 38.3, 36.2, 28.3, 21.3. ¹⁹F NMR (376 MHz, CDCl₃) δ –106.61. MS (ESI⁺): *m/z* 280.18 [M+H]⁺.

1-(4-Fluorophenyl)-5-(4-(hydroxymethyl)piperidin-1-yl)pentan-1-one (7h). Compound **12** (160 mg, 0.62 mmol), 4-piperidinemethanol (85 mg, 0.74 mmol), K₂CO₃ (102 mg, 0.74 mmol) and NaI in catalytic amounts were added into CH₃CN (25 mL) and afforded **7h** (98 mg, 54%). ¹H NMR (400 MHz, CDCl₃) δ 8.03–7.91 (m, 2H), 7.12 (t, J = 8.6 Hz, 2H), 3.52 (d, J = 5.7 Hz, 2H), 3.25 (d, J = 11.9 Hz, 2H), 3.01 (t, J = 6.5 Hz, 2H), 2.69 (t, J = 7.3 Hz, 2H), 2.37 (t, J = 11.6 Hz, 2H), 1.94–1.53 (m, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 198.3, 165.7, 133.2, 130.6, 115.7, 66.9, 58.0, 53.1, 37.9, 37.5, 27.5, 25.3, 21.8. ¹⁹F NMR (376 MHz, CDCl₃) δ –106.25. MS (ESI⁺): m/z 294.11 [M+H]⁺.

4-(1,4-Dioxa-8-azaspiro[4.5]decan-8-ylmethyl)phenol (13). The precursor of 5a was synthesized by a similar reported method.³⁵ Compound 9 (670 mg, 4.68 mmol) and

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4-hydroxybenzaldehyde (629 mg, 5.15 mmol) were dissolved in anhydrous dichloromethane (40 mL) and stirring at room temperature for 2 h. Then NaBH(OAc)₃ (1.48 g, 7.02 mmol) was added and the mixture was stirred at room temperature over night. After washing with saturated NaHCO₃ solution and extraction with dichloromethane, the combined organic layers were dried by Na₂SO₄. The solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography (petroleum ether:ethyl acetate = 1:2 (*v*/*v*)) to afford compound **13** (530 mg, 46%) as a colorless solid. ¹H NMR (400 MHz, CDCl₃) δ 7.08 (d, *J* = 8.5 Hz, 2H), 6.60 (d, *J* = 8.5 Hz, 2H), 3.94 (s, 4H), 3.48 (s, 2H), 2.59 (s, 4H), 1.78 (t, *J* = 5.7 Hz, 4H). ¹³C NMR(101 MHz, CDCl₃) δ 155.6, 131.0, 128.2, 115.3, 107.2, 64.2, 62.0, 50.9, 34.3. MS (ESI+) *m/z*: 250.14 [M + H]⁺.

Radioligand Competition Studies. The σ_1 assay was performed with the radioligand (+)-[³H]pentazocine (1288 GBq/µmol; PerkinElmer) using membrane homogenates obtained from rat cortex (SPRD, female, 10–12 weeks). The σ_2 assay was performed with the radioligand [³H]DTG (1761 GBq/µmol; PerkinElmer) using membrane homogenates obtained from rat liver (SPRD, female, 10–12 weeks). The VAChT assay was performed with radioligand (–)-[³H]-vesamicol (1 nM) using membrane homogenates from PC12 cells transfected with rat VAChT.

The procedures for the radioligand competition assays of 5a-5e, 6a, 6b and 7a-7h were previously described.^{35,40} Detailed procedures are presented in the Supporting Information.

Radiochemistry. The TRACERLabFX_{-FN} synthesis module (GE) was used for the production of $[^{18}F]$ **5a**. A cleaning program was carried out prior to the start of the radiosynthesis. The corresponding starting materials were stocked in the storage vessels and in the mixing flask. All reactions were performed with the starting $[^{18}F]$ fluoride activity of

5–15 GBq and the [¹⁸F]fluoride aqueous solution was trapped on the anion exchange cartridge (QMA, Waters) and eluted into the reaction vessel with a solution of Kryptofix 222 (15 mg, 40 μ mol), K₂CO₃ (2.7 mg, 20 μ mol) and Cs₂CO₃ (30 mg, 92.1 μ mol) in 1.5 mL of acetonitrile/water = 1:1 (ν/ν). The [¹⁸F]fluoride eluate was dried under vacuum, a nitrogen stream at 95 °C and addition of 3 × 1 mL of acetonitrile. After the completed drying, ethylene 1,2-ditosylate (6.0 mg, 16.2 mmol) in 0.64 mL of acetonitrile was added into the reaction vessel. The mixture was heated at 90 °C for 15 min. After cooling, hydroxyl precursor **13** (9.0 mg, 36.1 mmol) in 0.44 mL of DMF was added in the reaction vessel in succession. After heating at 110 °C for 10 min, the mixture was diluted with 1 mL of HPLC eluent and transferred to the semi-preparative HPLC with a flow of 5 mL/min. The fraction with a retention time about 20 min containing the radiotracer [¹⁸F]**5a** was transferred into the mixing flask containing 30 mL of water where the product was trapped by a reversed phase C18 cartridge, After washing with water, [¹⁸F]**5a** was eluted off with 1 mL of ethanol and diluted with sterile saline for *in vitro* and *in vivo* experiments.

Determination of log *D* **Value.** Flask shake method with 1-octanol/phosphate-buffered saline (PBS, 0.05 mol/L, pH = 7.4) was applied to determine the log *D* value of $[^{18}F]$ **5a** according to literature.^{35,40} The detailed procedure is shown in the Supporting Information.

In Vitro Stability. The *in vitro* stability of $[^{18}F]$ 5a was evaluated by monitoring the radiochemical purity at 2 hour. The procedures to determine the *in vitro* stability in ethanol, saline and human plasma were similar to those in the literature.^{35,40} The details are provided in the Supporting Information.

Cell Uptake. Cellular uptake experiments for evaluation of [¹⁸F]5a in vitro were performed

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in the following tumor cell models using a protocol published elsewhere with some modifications.⁵⁵ A375 (human malignant melanoma line; ATCC CRL-1619), PC3 (androgen-independent human malignant prostate adenocarcinoma line, ATCC CRL-1435), DU145 (androgen-independent human metastatic prostate adenocarcinoma line; ATCC HTB-81), and MCF7 (human metastatic mammary adenocarcinoma line; ATCC HTB-22) cells were used in this experiment. Substantial expression of σ -receptor subtypes σ_1 and σ_2 as well as binding of specific σ -receptor ligands has been demonstrated in the literature for all selected cell lines.^{19,56,57}

Cells were routinely cultivated as the reported method.⁵⁵ Cells were seeded in 24-well plates at a density of 1.3×10^5 cells/mL (A375, PC3, MCF7) and 1.5×10^5 cells/mL (DU145) and grown to confluence, The cell tracer uptake experiments using compound [¹⁸F]**5a** (0.4–0.7 MBq/mL; specific activity at application time: 30 GBq/µmol) were performed in quadruplicate in PBS at 37°C for 1, 10, 30, 60, and 120 min (2-3 independent experiments without blocking; 1-2 independent experiments with blocking). For blocking experiments, the cells were pre-incubated for 10 min with 10 µM of the following σ -receptor ligands: haloperidol, fluspidine or SA4503. The details of cell culture and cell uptake experiments are shown in the Supporting Information.

Generation of Mouse Tumor Xenografts. For the generation of subcutaneous tumors PC3, DU145, A375 and A431 (human epidermoid (squamous) cell carcinoma, ATCC CRL-1555) cells were used. The latter were cultivated following the same protocol reported above for cellular uptake experiments. Nine weeks old male and female NMRI (nu/nu) mice were subcutaneously xenotransplanted into the legs with these cells according to published protocol.^{58,59} The procedures are shown in the Supporting Information.

Biodistribution. The male Wistar rats (n = 32, Wistar Unilever, HsdCpb:WU, Harlan Winkelmann GmbH, Borchen, Germany) were between 7 and 9 weeks of age and body weight of 125 ± 14 g were used in the biodistribution study. The procedures are based on the reported literature and shown in the Supporting Information.⁵⁸⁻⁶¹

Metabolite Analysis. Metabolic stability studies were performed on male Wistar rats. Arterial blood samples were collected at 1, 3, 5, 10, 20, 30, and 60 min after injections of $[^{18}F]$ **5a**. Blood cells were separated by centrifugation (5 °C, 5 min, 8000 rpm), and plasma proteins were precipitated using 60% acetonitrile and subsequent centrifugation (5 °C, 5 min, 8000 rpm). The deproteinated supernatant was analyzed by radio-HPLC. The radio-HPLC system (Agilent 1100 series) applied for metabolite analysis was equipped with UV detection (254 nm) and an external radiochemical detector (Ramona, Raytest GmbH, Straubenhardt, Germany). Analysis was performed on a Zorbax C18 300SB (250 × 9.4 mm; 4 µm) column with an eluent system C (water + 0.1% TFA) and D (acetonitrile + 0.1% TFA) in a gradient 5 min 95% C, 10 min to 95% D, and 5 min at 95% D at a flow rate of 3 mL/min. HPLC were performed on arterial blood samples from 1 to 60 min after injections and on urine sample from 60 min after injection. The metabolite fraction in the blood plasma was calculated. The extracts from other tissues were prepared by ultrasound homogenization as 10% solution in PBS, centrifugation (5 °C, 70 000 g × min) and subsequent precipitation by 60% acetonitrile. The supernatant was analyzed by the described radio-HPLC.

Small Animal PET. Evaluation of tumor metabolism was performed by dynamic small animal positron emission tomography using the radiotracer [¹⁸F]**5a**. Transmission scans, CT and PET acquisition followed the protocol given by us in detail elsewhere.^{55,58,61} The procedures are shown in the Supporting Information.

Ex Vivo Autoradiography. Four adult male Wistar rats weighing 119–132 g were allowed free access of food and water. [¹⁸F]**5a** was injected in physiological saline of 0.5 mL of [¹⁸F]**5a** containing 40 MBq into two rats (control) and 1 mg/kg body weight haloperidol (blocked) was administered intravenously under anesthesia with a gas mixture of 10% desfluran, and 30% oxygen/air. After injection, anesthesia was discontinued for the 1 h incubation animals and for the animals with 5 min incubation time was the anesthesia continued. At 5 or 60 minutes after injection, the animals were sacrificed and the brains were quickly removed and frozen in powdered dry ice/isopentane. Coronal brain slices (10 μ m) were cut with a microtome-cryostat (CM 1850, Leica Instruments, Germany), thaw-mounted onto glass slides (SuperFrost, Menzel, Germany), air-dried for 60 min and exposed to imaging plates (BAS-SR; Fuji-Photo Film Co., Ltd., Tokyo) or stored at -20 °C for 12 h in standard x-ray cassettes. The autoradiograms were scanned by the bioimaging analyzer system (BAS 5000, FujiPhoto Film Co., Ltd.).

Statistical Analysis. Values are expressed as mean \pm SD or SE. Values were compared using an unpaired Student's *t*-test with Welch's correction and an *F*-test to compare the variances (GraphPad Prism 5.02 for Windows, GraphPad Software, San Diego, CA). The nonparametric Wilcoxon signed rank test and the D'Agostino-Pearson normality test were used for statistical evaluation for some of the data. *p* < 0.05 was considered significant and is indicated by an asterisk.

ASSOCIATED CONTENT

Supporting Information

General information and some parts of evaluation of [¹⁸F]5a in the Experimental Section,

radiochemical purity and biodistribution of $[^{18}F]$ **5a**, rat brain PET imaging and metabolic studies of $[^{18}F]$ **5a**, purities and the ¹H NMR of the final compounds. This material is available free of charge via the Internet at <u>http://pubs.acs.org</u>.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

AD, Alzheimer's disease; A_S, specific activity; AUCs, area under the curve values; BAT, brown adipose tissue; BBB, blood-brain barrier; [¹¹C]SA4503, 1-(3-methoxy-4-[¹¹C]methoxyphenethyl)-4-(3-phenylpropyl)piperazine; [¹⁸F]FPS,

1-3-[¹⁸F]fluoropropyl-4-((4-cyanophenoxy)-methyl) piperidine; [¹⁸F]ISO-1, *N*-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-2-(2-[¹⁸F]fluoroethoxy)-5-meth ylbenzamide; [³H]DTG, [³H]-1,3-di-*o*-tolyl-guanidine; HPLC, high performance liquid chromatography; HRMS, high resolution mass spectrometry; Hz, Hertz; ID, injected dose; %ID, percentage of injected dose; NSCLC, non small cell lung cancer; PBS, phosphate-buffered saline; PD, Parkinson's disease; PET, positron emission tomography; P-gp, P-glycoprotein; PGRMC1, progesterone receptor membrane component 1; RCP, radiochemical purity; r. t., room temperature; SUV, standardized uptake value; VAChT, vesicular acetylcholine transporter; WAT, white adipose tissue.

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Compd.	$K_{i}(\sigma_{1})$ (nM)	$K_i(\sigma_2)$ (nM)	$K_{i}(\sigma_{2})/K_{i}(\sigma_{1})$	
5a	5.4 ± 0.4	164 ± 20	30.4	
5b	7.3 ± 0.8	164 ± 7.8	22.5	
5c	5.3 ± 2.2	232 ± 29	43.8	
5d	3.3 ± 0.6	235 ± 24	71.2	
5e	10.1 ± 5.2	523 ± 31	51.8	
6a	5.0 ± 1.7	312 ± 73	62.4	
6b	11.2 ± 2.2	179 ± 16	16.0	
7a	4.0 ± 0.8	138.5 ± 16.0	34.6	
7b	3.5 ± 0.06	23.0 ± 9.1	6.6	
7c	23.8 ± 14.9	324 ± 57.3	13.6	
7d	15.4 ± 7.1	108 ± 6.4	7.0	
7e	146 ± 2.1	506 ± 41.7	3.5	
7f	19.8 ± 1.8	118 ± 18.4	6.0	
7g	284 ± 77.8	344 ± 7.8	1.2	
7h	152 ± 0.7	90.6 ± 8.9	0.6	
3 ^b	0.79 ± 0.11	277 ± 71	350	
SA4503 ^c	4.4 ± 1.0	242 ± 17	55	
SA4503 ^d	3.33 ± 0.12	50.7 ± 1.27	15.2	
Fluspidine ^e	0.59 ± 0.20	785	1331	

Table 1. Binding affinities of the piperidine ligands for σ_1 and σ_2 receptors ^a

^a Values are means \pm standard deviation (SD) of at least two experiments performed in triplicate.

^b From reference 35. ^c from reference 41. ^d from reference 40.^e from reference 33.



Figure 1. Representative structures of σ ligands used for PET imaging.



Figure 2. Design concept of target compounds 5a–5e, 6a, 6b and 7a–7h.



Figure 3. *In vitro* study on cellular association of [¹⁸F]**5a** in human tumor cells A375, PC3, DU145, and MCF7. Blocking experiments were performed by preincubation (for 30 min) with 10 μ mol/L of σ ligands haloperidol and/or fluspidine and/or SA4503. Results are given as percentage of injected dose (%ID) per mg protein (mean ± SD; n ≥ 8 for controls, n ≥ 4 for blocking experiments).



Figure 4. Biodistribution of $[^{18}F]$ **5a** (injected dose 8.9 ± 1.7 MBq/kg body weight) in Wistar rats (body weight 125 ± 14 g) after single intravenous injection at 5 and 60 min postinjection in control animals and with simultaneous injection of 1 mg/kg body weight of haloperidol. The values are given as SUV mean \pm SD.

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Sample	PET	Autoradiography		
Time p.i.	Brain	Brain		Muscle
Control (5 min)	e	a α	СР	
Blocked (5 min)				
Control (60 min)			•	6
Blocked (60 min)		0	0	
MRI				

Figure 5. Equivalent brain sections of the PET, MRI and autoradiographic study. For comparison are muscle sections of the identical animals shown. The blocked animals received simultaneously with the radiotracer haloperidol with 1 mg/kg body weight. Abbreviations: Cb = cerebellum, Ct = cortex, T = thalamus.



Figure 6. Comparison of the $[{}^{18}F]$ **5a** AUC in the brain of Wistar rats that received isotonic NaCl as vehicle (n = 2) and blocked animals that received cyclosporin A (n = 2, 5 mg/kg body weight).



Figure 7. Time-activity curves of $[{}^{18}F]$ **5a** in the tumor of PC3 control and haloperidol blocked, A431 control and blocked, A375 and DU145 control, and on the right site at the mean SUV data of the studies midframe time 50 min (mean 40 to 60 min). *** p < 0.05.



Figure 8. Maximum intensity projections of a dynamic PET experiment with $[{}^{18}F]$ **5a** in an A431 tumor bearing NMRI *nu/nu* mouse (major organs for ${}^{18}F$ -activity accumulation: br, brain; li, liver; in, intestine; gs, submandibular glands; bl, bladder; and tu, tumor) at (**A**) 3 min (summarized from 1 to 5 min) and (**B**) 50 min (summarized from 40 to 60 min) postinjection. The image **C** represents the difference of both primary images (A and B) demonstrating enhanced $[{}^{18}F]$ **5a** regional accumulation in the A431 tumor as well as intestine and bladder. Additionally, representative difference images of dynamic PET experiments with $[{}^{18}F]$ **5a** in (**D**) A375, (**E**) PC3, and (**F**) DU145 tumor bearing NMRI *nu/nu* mice are given. The used PC3 animal model comprised mice with two xenotransplanted tumors.





Reagents and reaction conditions: (a) K₂CO₃, NaI, DMF, 90 °C, overnight, 58% (b) AIBN, NBS, CCl₄, 90 °C, 4 h, 67% (c) K₂CO₃, NaI, CH₃CN, reflux, 4 h, for **5a**, **8**, 54%; for **5b**, 4-fluorobenzyl bromide, 59%; for 5c, 4-iodobenzyl bromide, 62%; for 5d, 4-bromobenzyl bromide, 87%; for 5e, 4-methoxybenzyl bromide, 71%; for **6a**, 3-bromo-1-(4-fluorophenyl)propan-1-one, 39%; for **6b**, 4-bromo-1-(4-fluorophenyl)butan-1-one (11), 45%.





Reagents and reaction conditions: (a) K₂CO₃, NaI, CH₃CN, reflux, 4 h, for **7a**, **11** and **10a**, 69%; for **7b**, 5-bromo-1-(4-fluorophenyl)pentan-1-one (**12**) and **10a**, 63%; for **7c**, **11** and **10b**, 69%; for **7d**, **12** and **10b**, 42%; for **7e**, **11** and **10c**, 66%; for **7f**, **12** and **10c**, 52%; for **7g**, **11** and **10d**, 62%; for **7h**, **12** and **10d**, 54%.

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Reagents and reaction conditions: (a) NaBH(OAc)₃, DCM, r.t., overnight, 46% (b) $[^{18}F]F$, K_{2.2.2}, K₂CO₃, Cs₂CO₃, CH₃CN, 90 °C, 15 min (c) Cs₂CO₃, **13**, DMF, 110 °C, 10 min.

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