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Chiral resolution of serial potent and selective σ_1 ligands and biological evaluation of (–)-[^{18}F]TZ3108 in rodent and the nonhuman primate brain

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Abstract

Twelve optically pure enantiomers were obtained using either crystallization or chiral high performance liquid chromatography (HPLC) separation methodologies to resolve six racemic sigma-1 (σ_1) receptor ligands. The *in vitro* binding affinities of each enantiomer for σ_1 , σ_2 receptors and vesicular acetylcholine transporter (VACHT) were determined. Out of the 12 optically pure enantiomers, five displayed very high affinities for σ_1 ($K_i < 2$ nM) and high selectivity for σ_1 versus σ_2 and VACHT (>100-fold). The minus enantiomer, (–)-**14a** ((–)-**TZ3108**) ($K_{i-\sigma_1} = 1.8 \pm 0.4$ nM, $K_{i-\sigma_2} = 6960 \pm 810$ nM, $K_{i-VACHT} = 980 \pm 87$ nM), was chosen for radiolabeling and further *in vivo* evaluation in rodents and nonhuman primates (NHPs). A biodistribution study in Sprague Dawley rats showed brain uptake (%ID/gram) of (–)-[^{18}F]**TZ3108** reached 1.285 ± 0.062 at 5 min and 0.802 ± 0.129 at 120 min. NHP microPET imaging studies revealed higher brain uptake of (–)-[^{18}F]**TZ3108** and more favorable pharmacokinetics compared to its racemic counterpart. Pretreatment of the animal using two structurally different σ_1 ligands significantly decreased accumulation of (–)-[^{18}F]**TZ3108** in the brain. Together, our *in vivo* evaluation results suggest that (–)-[^{18}F]**TZ3108** is a promising positron emission tomography (PET) tracer for quantifying σ_1 receptor in the brain.

Graphical Abstract

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Keywords

Fluorine-18; Enantiomer; σ_1 receptor; PET imaging

1. Introduction

Sigma (σ) receptors are recognized as a non-opioid receptor family and have their own specific bioactivity binding patterns; they have a characteristic anatomical expression, and unique properties and modulation functions in the central nervous system (CNS).^{1, 2} They are also present in the endocrine and immune systems, and extensively distribute in both the brain and peripheral tissues, where they play an important role in cellular and organ processes including immunoregulation, motor and endocrine function, modulation of proliferation, and regulation of ion channels.^{2, 3} Two distinct σ receptor subtypes, termed σ_1 and σ_2 , have been characterized based on pharmacological data.⁴⁻⁶ The σ_1 receptor is a 29 kDa single polypeptide located on the outer membranes of cells; it is a small transmembrane protein cloned in 1996 which consists of 223 amino acids. It has no homology with any other reported mammalian receptor protein.⁷ The σ_1 receptor plays a pivotal role in many neurological disorders such as schizophrenia, cognition dysfunction, and addiction. Postmortem autoradiograph studies, and PET imaging studies showed reduced σ_1 receptor density in the brains of patients with Parkinson's and Alzheimer's diseases.⁸⁻¹⁰ Various σ_1 ligands are recognized as potent neuromodulators for various excitatory neurotransmitter systems including cholinergic, dopaminergic, and glutamatergic systems.¹¹

Positron emission tomography (PET) is a well-established imaging modality that is able to provide functional information by quantitatively assessing the changes of neurotransmitter receptors, transporters and enzymes in brain regions of living subjects.^{12, 13} PET with suitable σ_1 receptor radiotracer will provide a noninvasive methodology for quantifying the

expression levels of σ_1 receptor in animals and will be a useful tool in diagnosis of diseases at early stage, guiding the development of therapeutics targeting σ_1 receptor.¹ Tremendous efforts have been put into identification of a clinically suitable σ_1 PET radiotracer.¹⁴ Among the lead σ_1 radiotracers, 1-(4-methoxy-3-(methoxy-¹¹C)phenethyl-¹¹C)-4-(3-phenyl-propyl)piperazine ([¹¹C]SA4503, Figure 1)^{15, 16} is the most widely evaluated PET radioligand in humans. [¹¹C]SA4503 has been used to map σ_1 receptor expression in the brains of healthy controls and patients with Alzheimer's disease, Parkinson's disease, and other diseases, providing valuable information on σ_1 distribution in the brain under different conditions. Although SA4503 was reported to have high binding affinity for emopamil binding protein (EBP) ($K_i = 1.7$ nM)¹⁷ and moderate binding affinity for vesicular acetylcholine transporter (VACHT) ($K_i = 50$ nM),¹⁸ subsequent *in vivo* study in mice suggested the contribution of EBP binding of [¹¹C]SA4503 was negligible in the brain, probably due to a low EBP density. Blocking experiments using four σ_1 specific agents and two EBP specific agents confirmed the σ_1 selective binding of [¹¹C]SA4503 in the brain.¹⁹ [¹¹C]SA4503 displayed less than 100-fold selectivity over both σ_2 receptor and VACHT (55-fold for σ_2 , 11-fold for VACHT respectively).²⁰ Furthermore, the subcellular localization of σ_1 receptors seems similar to that of σ_2 receptors.²¹ These factors may cause higher *in vivo* non-specific binding and impact the accuracy in quantifying the density of σ_1 receptors *in vivo*.^{17, 22, 23} In addition, the slow brain washout kinetics of [¹¹C]SA4503 require at least a 90-min dynamic scan for a stable quantitative analysis; long scanning protocols using a ligand labeled with a radionuclide with a short half-life like carbon-11 ($t_{1/2} = 20.3$ min) places a high strain on both clinical data collection and the comfort of patients. Recently, ¹⁸F-6-(3-fluoropropyl)-3-(2-(azepan-1 yl)ethyl)benzo-[d]thiazol-2(3*H*)-one ([¹⁸F]FTC-146, Figure 1) ($K_{i-\sigma_1} = 2.5$ pM, $K_{i-\sigma_2} = 364$ nM, $K_{i-VACHT} = 450$ nM) was reported;²⁴ results using σ_1 receptor knock-out mice and wild type mice suggested that [¹⁸F]FTC-146 is a specific radiotracer for σ_1 receptor. Currently [¹⁸F]FTC-146 is under a phase 0 clinical trial study to test if [¹⁸F]FTC-146, is a suitable PET tracer for identifying the site of nerve injury/inflammation in response to painful conditions. The broad objectives of this clinical trial study included three components: dosimetry study of [¹⁸F]FTC-146 in human subjects; safety evaluation of [¹⁸F]FTC-146 in human subjects; evaluation of the pharmacokinetics and its ability of this radiotracer to map σ_1 expression in healthy subjects and specific patient subpopulations. The estimated complete of this phase 0 study is 2021.²⁵ Notwithstanding, it is not yet known if [¹⁸F]FTC-146 is a clinically suitable PET tracer for quantifying σ_1 receptor in the human brains.²⁶

In our search for a clinically suitable σ_1 PET tracer, we previously reported a series of carbonyl containing racemic prezamicol analogues (**14a–14f**, Figure 1)²⁷ with high σ_1 binding affinity and high selectivity for σ_1 over σ_2 and VACHT ($K_{i-\sigma_1}$ ranging from 0.48 to 26 nM, >100-fold selectivity for σ_1 over both σ_2 and VACHT). These racemic compounds were made by replacing the 4-phenylpiperidiny group in prezamicol with a 4-substituted benzoylpiperidiny group and alkylating the secondary amine in (3'-hydroxy-1,4'-bipiperidin-4-yl)(4-substituted phenyl)methanone. These newly developed compounds are structurally similar to the prezamicol scaffold. We also reported our preliminary evaluation of a representative racemic compound, [¹⁸F]**14a** (**TZ3108** ($K_{i-\sigma_1} = 0.48$ nM, > 1000-fold for σ_1 versus σ_2 and VACHT). [¹⁸F]**TZ3108** had high uptake in the brains of both rats and

nonhuman primates (NHPs); the uptake of [^{18}F]TZ3108 in the brain of nonhuman primates achieved steady state at 45 min post-injection; negligible radioactive metabolite crossed the blood brain barrier.²⁸ Slow clearance kinetics of [^{18}F]TZ3108 from σ_1 -enriched brain regions was observed, which could interfere with tracer modeling and *in vivo* quantification of σ_1 density. Furthermore, it is widely accepted that σ_1 receptor binding to compounds has stereoselectivity and σ_1 binding affinity distinguishes between enantiomers,^{29, 30, 31, 32, 33} for example recent reported enantiomeric σ_1 receptor ligands, (*R*)-(+)- and (*S*)-(-)- ^{18}F -fluspidine, showed distinctive *in vivo* kinetics in porcine brain.³² Based on our previous *in vivo* evaluation of racemic [^{18}F]TZ3108 and our effort in search of radiotracers for improving washout kinetics in the brain, with structural similar potent ligands, the less potent ligand has fast clearance kinetic than the more potent ligand from its binding sites. Therefore, the less potent minus enantiomer (-)-[^{18}F]TZ3108 was selected for radiosynthesis and evaluation *in vivo* in rodents and nonhuman primates. This study was inspired by: (1) σ_1 receptor ligands may be potent therapeutic drugs for the treatment of neurological disorders and other diseases; (2) identification of a clinically suitable ^{18}F -labeled radiotracer for quantitative imaging of the σ_1 receptor is imperative for assessment of CNS disorders; and (3) our previous effort suggested that further investigation of radiolabeled carbonyl containing prezamicol radiotracers has high possibility of identifying a clinically suitable σ_1 receptor PET radiotracer for human use.^{27, 28}

2. Results

2.1. Chemistry and radiochemistry

Chemistry—The racemic σ_1 receptor compounds **14a–14f**, and the radiolabeling via the nitro precursor (-)-TZ3138 for (-)-[^{18}F]14a ((-)-[^{18}F]TZ3108) were synthesized according to our reported procedures, with the regioselective ring-opening of the epoxy as a key step.^{27, 28} The chiral resolutions of the racemic compounds **14a–14f** and TZ3138 were accomplished using two different methodologies: a) selective crystallization, b) normal phase Chiralcel OD high performance liquid chromatography (HPLC) separation. Crystallization, a highly efficient and cost-effective methodology is the first choice. Fortunately, using enantiopure di-*p*-toluoyl tartaric acid (DTTA) as the chiral selector,³⁴ the enantiomers of racemic compounds **14a–c**, and **14e** were successfully separated by careful crystallization as described in the experimental section. In general, the crystallization process was performed twice for each enantiomer to achieve higher optical purity. However, for racemic compounds **14d**, **14f**, and TZ3138, crystallization was not able to separate the enantiomers, thus the chiral HPLC system was used for separation of the enantiomers. The structures of compounds **14d**, **14f**, and precursor TZ3138 are similar to other VAcHT compounds^{35, 36} possessing a hydroxyl group on the chiral center. A semi-preparative Chiralcel OD column was chosen for resolving these compounds. Typically, a mixture of hexane and 2-propanol was chosen as the mobile phase and trace amount of triethylamine (0.2% of the mobile phase) was added to reduce peak tailing.

The optical purity of each enantiomer was determined using analytic HPLC system equipped with an analytical Chiralcel OD column. The enantiomeric excess (*ee*) of each purified isomer was greater than 95%. The characteristic data (^1H NMR, ^{13}C NMR) for each

purified enantiomer was identical to its purified enantiomeric counterpart. Figure 2 shows the confirmation chromatograms for isolated precursor (–)-**TZ3138** and nonradiolabeled standard isomer (–)-**TZ3108**.

Radiochemistry—The radiosynthesis of (–)-[¹⁸F]**TZ3108** was accomplished by the nucleophilic aromatic substitution of a nitro-containing precursor (–)-**TZ3138** with [¹⁸F]fluoride (Scheme 1). The desired product was separated from precursor (–)-**TZ3138** and a side product using a reversed phase semi-preparative HPLC column under optimized conditions. The production of (–)-[¹⁸F]**TZ3108** was achieved in 19 ± 2% yield, with a specific activity of 58 – 84 GBq/μmol (n > 10, decay corrected to the end of synthesis).

2.2. *In vitro* binding assay

The affinities of each enantiomer binding to σ_1 , σ_2 , and VACHT were determined according to the published procedures using a radioactive competitive screening assay.^{27, 36, 37} Our *in vitro* screening results of the 12 enantiomers are shown in Table 1. For compounds **14a–f**, the minus enantiomer and its counterpart plus enantiomer did distinguish their potency toward σ_1 receptor; the (+)-isomers of **14a–f** are 2.6 – 37 fold more potent than the corresponding (–)-isomers and 1.1 – 8.6 fold more potent than the racemic ligands. The data clearly demonstrated stereoselective binding to the σ_1 receptor. All 12 enantiomers have very weak *in vitro* binding to σ_2 and VACHT, resulting in high selectivity for σ_1 versus σ_2 or VACHT. Among all 12 enantiomers, compounds (+)-**14a–c&e**, (–)-**14a** are very potent binding to σ_1 receptor ($K_i < 2$ nM), exhibiting high selectivity for σ_1 versus σ_2 and VACHT (both σ_1 /VACHT and $\sigma_1/\sigma_2 > 130$ -fold). Of the five most potent enantiomers, (+)-**14a** ((+)-**TZ3108**) displayed over 3-fold more potency than the racemic counterpart **TZ3108**, and 13-fold more potency than its enantiomer (–)-**TZ3108** for σ_1 receptor.

2.3. *Ex vivo* biodistribution in rats

The *ex vivo* biodistribution study of (–)-[¹⁸F]**TZ3108** was performed using male Sprague Dawley rats euthanized at 5, 30, 60 and 120 min post intravenous administration of 2.1 – 2.4 MBq of the radiotracer. The tissue distribution data are presented in Table 2. (–)-[¹⁸F]**TZ3108** rapidly penetrates the blood brain barrier and accumulates in the rat brain. The brain uptake (%ID/g) of (–)-[¹⁸F]**TZ3108** reached 1.285 ± 0.062 at 5 min and remained 0.864 ± 0.092 at 60 min, demonstrating that (–)-[¹⁸F]**TZ3108** has a good uptake and high retention in rat brain. Compared to the brain uptake of racemic [¹⁸F]**TZ3108** (1.586 ± 0.505 , 1.321 ± 0.082 at 5 and 60 min respectively, $K_i = 0.48$ nM for σ_1 receptor), the lower potency of (–)-[¹⁸F]**TZ3108** ($K_i = 1.8$ nM for σ_1 receptor) may contribute the slightly faster washout of (–)-[¹⁸F]**TZ3108**;²⁸ the minor lower brain uptake of (–)-[¹⁸F]**TZ3108** may resulted from either the variability of the biodistribution experiment or the lower binding affinity of (–)-[¹⁸F]**TZ3108** to σ_1 receptor. Nevertheless, relatively high uptake (%ID/g) of (–)-[¹⁸F]**TZ3108** observed in multiple brain regions including cerebellum, cortex, thalamus, hippocampus with the highest uptake (%ID/g) in cortex (1.652 ± 0.067) and lowest uptake in striatum (1.021 ± 0.057) at 5 min. (–)-[¹⁸F]**TZ3108** gradually washed out the brain but was still remained relatively high at 120 min in all brain regions; the regional brain uptake was 0.877 ± 0.189 , 0.684 ± 0.107 , 0.810 ± 0.149 , 0.790 ± 0.108 at 120 min in cortex, striatum,

thalamus, and hippocampus respectively. Lower bone uptake (%ID/g) of (–)-[¹⁸F]TZ3108 was observed at 5 min (0.32 ± 0.01) with negligible change until 120 min (0.32 ± 0.03). A low blood uptake (%ID/g) of 0.051 ± 0.017 at 5 min and 0.013 ± 0.003 at 120 min was observed. For peripheral tissues, high uptakes in liver, spleen, and pancreas were observed at all time points.

2.4. MicroPET studies in nonhuman primate

MicroPET studies of (–)-[¹⁸F]TZ3108 were performed in the brain of an adult cynomolgus macaque. Representative whole brain PET/MRI images and standardized uptake values (SUV) are presented in Figure 3, indicating that (–)-[¹⁸F]TZ3108 rapidly entered the monkey brain and peaked at 30 min post-injection. (–)-[¹⁸F]TZ3108 displayed high uptake in the frontal cortex, striatum, thalamus, hippocampus, cerebellum. The distribution of (–)-[¹⁸F]TZ3108 was consistent with the σ_1 receptor distribution in NHP brain.^{26, 38–40} The washout from the cerebellum was slightly faster than that from frontal cortex, thalamus and hippocampus. Cerebellum is a σ_1 enriched region in the brain. Similar kinetics were reported in the frontal cortex, cerebellum, and hippocampus of conscious monkeys administered a σ_1 -specific PET tracer.⁴⁰ The regional brain uptake reached a plateau in most brain regions of interest at ~30 min post-injection. Compared to racemic [¹⁸F]TZ3108, which required 45 min to reach the steady state, (–)-[¹⁸F]TZ3108 had an improved kinetics in most σ_1 -enriched regions, such as cerebellum and frontal cortex.²⁸

To confirm the *in vivo* binding specificity of (–)-[¹⁸F]TZ3108, the NHP was pretreated with a potent σ_1 ligand, *N*-(4-benzyl cyclohexyl)-2-(2-fluorophenyl)acetamide, Yun122^{28, 41} ($K_{i-\sigma_1} = 3.6$ nM, $K_{i-\sigma_2} = 667$ nM) 5 min prior to tracer injection. The PET study results revealed that the brain uptake of (–)-[¹⁸F]TZ3108 was significantly reduced in the pretreated animal compared to the baseline condition. As shown in Figure 4, Yun122 caused obvious reduction in the uptake of (–)-[¹⁸F]TZ3108 in the frontal cortex, striatum, thalamus, hippocampus, and cerebellum; among these regions, the cerebellum had the most reduction of tracer uptake; the hippocampus had the least reduction of tracer uptake upon Yun122 treatment.

Because [¹¹C]SA4503 has been widely used for clinical investigations of σ_1 receptor expression levels, additional PET studies of (–)-[¹⁸F]TZ3108 were performed in a macaque pretreated with SA4503 at a dose of 1.5 mg/kg following the same procedure for pretreating the animal with Yun122. Clear reduction of (–)-[¹⁸F]TZ3108 uptake in the brain was observed, but the decrease resulting from use of SA4503 was less than that caused by Yun122, as shown in Figure 4. However, the cerebellum had the greatest uptake reduction upon pretreatment using both σ_1 -specific compounds. Slight change was observed in the thalamus and hippocampus for SA4502 pretreated animal. The higher decrease of the tracer uptake caused by Yun122 may be due to the slightly higher σ_1 binding potency of Yun122 than that of SA4503. Altogether, our microPET imaging data suggest that (–)-[¹⁸F]TZ3108 has *in vivo* σ_1 receptor binding specificity.

3. Discussion

A series of studies suggests that σ_1 receptor is involved in modulating several neurotransmitter systems including cholinergic, glutamatergic, and serotonergic neurotransmission.^{42–44} Aberrant expression levels of σ_1 receptor play important roles in neurodegenerative diseases such as major depression, schizophrenia, and brain ischemia.¹⁸ A PET radiotracer with high binding potency and high selectivity for σ_1 over other neuroreceptors (σ_2 , VACHT and others), and suitable *in vivo* pharmacokinetics would provide a useful tool to assess change of σ_1 receptor expression in the brains of patients with CNS disorders. However, lack of a highly selective σ_1 ligand and the unfavorable *in vivo* kinetics among the current generation of σ_1 receptor radiotracers continually impel investigators to put tremendous effort into identifying a clinically suitable PET tracer for imaging σ_1 receptors. Fluorine-18 has a favorable half-life of 109.8 min, enables multiple-step radiosynthesis and provides convenience in clinical acquisition of imaging data, permits delivery of an ¹⁸F-labeled radiopharmaceutical to a satellite clinical PET center within suitable driving distance, and allows multiple scans of patients in the same day with one batch of fluorine-18 production. Therefore, in recent years identification of an ¹⁸F-labeled clinical radiotracer suitable for σ_1 receptor imaging by PET in living subjects attracts considerable attention.

It is widely accepted that σ_1 receptor has stereoselective binding. For example, the (+)-enantiomers of benzomorphan derivatives, including pentazocine, dextrallorphan, and cyclazocine, bind with high affinity to the σ receptor whereas the (–)-enantiomers bind to the classical opioid receptors.^{45, 46} Consequentially, resolving promising σ_1 racemic ligand to perform the *in vitro* and *in vivo* evaluation of enantiomeric radiotracers will have high possibilities of success in identification of clinically suitable σ_1 PET radiotracers.^{31, 47} As reported, chiral separation of vesamicol derivatives is a very challenging task although detailed information including chiral stationary phase, mobile phase, and flow rate was reported and discussed.^{48, 49} Out of six racemic σ compounds, four compounds **14a–c** and **14e** could be easily separated using a crystallization procedure as described in the experimental section. The other racemic compounds **14d** and **14f** were not able to be resolved by crystallization although we had tried different solvent systems and chiral selectors. To overcome this challenge, a normal phase Chiralcel OD HPLC column was used to resolve compounds **14d**, **14f** and also **TZ3138** under optimized HPLC conditions.

Our *in vitro* binding data of the 12 enantiomers showed that both minus and plus isomers of each racemic compound exhibit distinguishing σ_1 binding affinity; each enantiomer also exhibits a difference in σ_1 receptor binding potency relative to the corresponding racemic compound. The highly potent enantiomers with reasonable binding affinity difference offer the possibility to evaluate a panel of radiotracers with different *in vivo* binding kinetics of radiotracers in the brain and to identify a σ_1 radiotracer having favorable clearance kinetics from brain. A suitable σ_1 PET radiotracer must have high specificity of binding sites in the brain and be able to achieve steady state within short time post-injection and have fast clearance from target regions. This will allow the image data acquisition in a reasonable time window and reduce patient distress in clinical practice. We previously reported that [¹⁸F]**TZ3108** had good brain accumulation and specific binding to σ_1 receptor in the brains

of rodents and NHPs. However, the clearance kinetics of [^{18}F]TZ3108 from the target brain regions was relatively slow. Tracer uptake reached peak at 45 min and maintained itself at almost the same level for > 120 min in the σ_1 -enriched regions of the brain. Although both the minus and plus enantiomers of TZ3108 showed good binding affinities and excellent selectivity ($K_{i-\sigma_1} < 2$ nM, selectivity > 500-fold for σ_1 over σ_2 receptors and VACHT), the minus isomer, (-)-TZ3108, is about 4-fold less potent than the racemic TZ3108. A radiotracer with lower affinity may have faster clearance kinetics from target regions than a higher affinity radiotracer with similar structure and physicochemical properties. This strategy has been implemented for development of PET tracers on different targets such as σ_1 receptor,^{50, 51} phosphodiesterase 10A (PDE10A),⁵² and amyloid- β protein.⁵³ Therefore, we chose to radiosynthesize the less potent isomer (-)-[^{18}F]TZ3108 to test its *in vivo* suitability to be a σ_1 receptor PET radiotracer.

For radiosynthesis of (-)-[^{18}F]TZ3108, it was found that higher temperature was required for the aromatic nucleophilic substitution reaction because an electron rich methylene group was located in the para-position of the nitro leaving group. However, decomposition of the precursor under higher temperature was observed. A compromised strategy using stepwise addition of the precursor to the reaction vessel was employed to achieve higher yield. Briefly, a solution of (-)-TZ3138 (2–3 mg) in dimethyl sulfoxide (DMSO) was made; half of the solution was added into the fluorine-18 containing vessel and heated 10 min at 125 °C as detailed in the experimental section, then the second portion of the solution of (-)-TZ3138 was added and heated an additional 10 min at the same temperature. For the HPLC purification of the radioactive product, to achieve high chemical purity of (-)-[^{18}F]TZ3108 without nitro precursor contamination, we optimized the mobile phase condition (20% acetonitrile in 80% 0.1 M ammonium formate buffer, pH ~4.5) to allow the radioactive product peak to be eluted 1 min prior to its nitro precursor (-)-TZ3138. Analytical HPLC demonstrated that our radiochemical procedure was able to deliver the product of (-)-[^{18}F]TZ3108 in good yield and high radiochemical purity.

Biodistribution results using Sprague Dawley rats suggested that (-)-[^{18}F]TZ3108 quickly entered the brain and had high brain uptake. (-)-[^{18}F]TZ3108 also displayed higher uptake in the σ_1 -enriched peripheral tissues including pancreas, spleen, kidney, and liver. From 5 to 120 min, negligible uptake in blood and bone was observed, indicating (-)-[^{18}F]TZ3108 has low binding to plasma proteins and negligible defluorination *in vivo*, distinguished from [^{18}F]FTC-146 for which radioactivity accumulation was reported in the skull of monkey at 60 min post-injection.²⁶ Compared to the microPET imaging results of racemic [^{18}F]TZ3108 ($K_{i-\sigma_1} = 0.48$ nM) in monkeys, the less potent (-)-[^{18}F]TZ3108 ($K_{i-\sigma_1} = 1.8$ nM) did display more rapid clearance kinetics from the brain, suggesting that the slight reduction in binding affinity improved clearance kinetics of the radiotracer brain uptake of (-)-[^{18}F]TZ3108 peaked at 30 min post-injection, in contrast to 45 min for racemic [^{18}F]TZ3108. Using either Yun122 or SA4503 to pretreat the animal significantly reduced the uptake of (-)-[^{18}F]TZ3108, in most brain regions including cerebellum, striatum, and frontal cortex. The different reduction of SUV may result from the fact that Yun122 is more potent than SA4503 for σ_1 receptor.

Together, (–)-[¹⁸F]TZ3108 is a potent and specific σ_1 radiotracer, and it has favorable *in vivo* clearance kinetics from its binding sites in the brain. At 30 min post-injection, the brain uptake of (–)-[¹⁸F]TZ3108 reached its peak compared to 45 min for [¹⁸F]TZ3108. In addition, (–)-[¹⁸F]TZ3108 is a more potent tracer than [¹¹C]SA4503 for σ_1 receptor yet has much more favorable *in vivo* kinetics. (–)-[¹⁸F]TZ3108 was easily made by [¹⁸F]F[–]/nitro displacement reaction. A fluorine-18 radiotracer can be distributed to satellite clinical sites, requires less time for production, and allows clinical data collection for longer time period. Therefore, our findings suggest that (–)-[¹⁸F]TZ3108 has potential to be a suitable PET tracer for quantitative imaging of σ_1 receptor expression levels *in vivo*.

4. Conclusions

We successfully resolved 6 racemic σ_1 receptor ligands to obtain enantiomeric minus and plus isomers. *In vitro* competitive binding assay showed that five of the 12 optically pure enantiomers displayed very high affinities and selectivity for σ_1 receptor over σ_2 receptor and VACHT ($K_{i-\sigma_1} < 2$ nM, selectivity for σ_2 and VACHT > 100 -fold). One of the potent enantiomers (–)-[¹⁸F]TZ3108 was radiosynthesized with a good radiochemical yield and high specific activity. *Ex vivo* biodistribution in rodents and *in vivo* micro PET scans in NHPs demonstrate that (–)-[¹⁸F]TZ3108 is highly specific and selective for σ_1 receptor with favorable clearance kinetics compared to its racemic counterpart [¹⁸F]TZ3108, suggesting (–)-[¹⁸F]TZ3108 is a promising PET tracer for quantifying σ_1 receptor levels in the brain of living subjects. Further performance of the dosimetry and toxicity evaluations will guarantee the translation of this tracer into clinical evaluation of its suitability to quantify changes in σ_1 receptors in humans.

5. Experimental

5.1. General

All solvents and reagents were obtained commercially and used as received. ¹H NMR spectra were recorded on a Varian Mercury-VX 300 or 400 spectrometer. The chemical shifts are reported as δ values (ppm) relative to tetramethylsilane (TMS) as an internal reference. The SpectraSystem was used for both analytical and semi-preparative HPLC of radiolabeling production and quality control. A Chiralcel OD normal phase HPLC column was used to resolve enantiomers. The specific optical rotation was determined on an automatic polarimeter (Autopol 111, Rudolph Research, Flanders, NJ). [¹⁸F]Fluoride was produced from a RDS111 cyclotron (Siemens/CTI Molecular Imaging, Knoxville, TN) by ¹⁸O(p, n)¹⁸F reaction through proton irradiation of enriched ¹⁸O water (95%). [¹⁸F]Fluoride was first passed through an ion-exchange resin then eluted using 0.02 M potassium carbonate (K₂CO₃) solution.

5.2. Chemistry

The synthesis of racemic **14a–f** and radiolabeling precursor **TZ3138** were conducted according to our previously published procedures.²⁷ The racemic compounds and radiolabeling precursor for (–)-[¹⁸F]TZ3108 were resolved either by recrystallization or a Chiralcel OD column.

5.2.1. (1'-(4-Fluorobenzyl)-3'-hydroxy-[1,4'-bipiperidin]-4-yl)(4-fluorophenyl)methanone, (+)-TZ3108, ((+)-14a)—A solution of (±)-TZ3108 (100 mg, 0.24 mmol) in acetone (2 mL) was treated with a solution of (+)-di-p-toluoyl-D-tartaric acid (98 mg, 0.24 mmol) in acetone (2 mL). The mixture was left at room temperature to crystallize and the resulting crystals were collected by filtration and washed with cold acetone (82 mg, *ee* 90.5% for released free base). These crystals were then partitioned between 1 M NaOH(aq.) and CH₂Cl₂. After separation of the CH₂Cl₂ layer, the aqueous layer was extracted with CH₂Cl₂. The combined organic extracts were washed with saturated Na₂CO₃ and brine solution, and dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The above resolution process was repeated again, and collectively 28 mg of (+)-TZ3108 (*ee* 95.5%) (Chiral HPLC analytical conditions: Chiralcel OD column, 4.6 mm × 250 mm, eluting composed of 94.8% Hexane + 5% 2-propanol + 0.2% Et₃N, flow rate 1 mL/min, oven temperature 25 °C, detection UV 254 nm). ¹H NMR (300 MHz, CDCl₃): δ 1.62 (q, *J* = 12.0 Hz, 1H), 1.77 (d, *J* = 11.7 Hz, 2H), 1.84–1.90 (m, 4H), 1.99 (t, *J* = 12.9 Hz, 1H), 2.26 (q, *J* = 12.8 Hz, 2H), 2.71–2.76 (m, 2H), 2.95 (t, *J* = 13.2 Hz, 2H), 3.17–3.25 (m, 2H), 3.50 (s, 2H), 3.55–3.63 (m, 1H), 6.99 (t, *J* = 8.8 Hz, 2H), 7.13 (t, *J* = 8.7 Hz, 2H), 7.25 (d, *J* = 8.1 Hz, 2H), 7.96 (td, *J* = 2.7, 10.2 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 21.8, 29.5, 29.6, 44.0, 45.5, 52.0, 53.1, 59.0, 62.1, 66.2, 69.8, 115.0, 115.3, 115.8, 116.1, 130.7, 130.8, 131.0, 131.1, 201.1. The optical rotation of (+)-14a was [*α*]_D²⁰ = +7.3° (1.1 mg/mL in MeOH). HRMS (ESI) Calcd for C₂₄H₂₉F₂N₂O₂ [M+H]⁺ 415.2192, found: 415.2193. (+)-TZ3108 was converted to its oxalate salt according to reported procedure. (+)-TZ3108 oxalate, mp: 210 °C (decomposed).

5.2.2. (1'-(4-Fluorobenzyl)-3'-hydroxy-[1,4'-bipiperidin]-4-yl)(4-fluorophenyl)methanone, (–)-TZ3108 ((–)-14a)—The acetone mother liquid from the above resolution was evaporated to dryness to give oil. The oil was portioned between 1 M NaOH and CH₂Cl₂. After separation of the CH₂Cl₂ layer, the aqueous layer was extracted with CH₂Cl₂. The combined organic extracts were washed with saturated Na₂CO₃ and brine solution, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure to afford a white solid that was again dissolved in acetone (2 mL), and a solution of (–)-di-p-toluoyl-L-tartaric acid (96.2 mg, 0.24 mmol) in acetone (2 mL) was added. The mixture was set aside to crystallize at room temperature. The crystals were collected by filtration and washed with cold acetone (79 mg, *ee* 93.5% for released free base). The above resolution process was repeated again, and collectively 25 mg of (–)-TZ3108 (*ee* 95.1%), Chiral HPLC analytical conditions: Chiralcel OD column, 4.6 mm × 250 mm, eluting with 94.8% Hexane + 5% 2-propanol + 0.2% Et₃N, flow rate 1 mL/min, oven temperature 25 °C, detection UV 254 nm). ¹H NMR (300 MHz, CDCl₃): δ 1.55 (q, *J* = 12.2 Hz, 1H), 1.73 (d, *J* = 11.9 Hz, 2H), 1.84–1.90 (m, 4H), 1.99 (t, *J* = 12.7 Hz, 1H), 2.25 (q, *J* = 12.0 Hz, 2H), 2.67–2.75 (m, 2H), 2.95 (t, *J* = 14.7 Hz, 2H), 3.17–3.27 (m, 2H), 3.50 (s, 2H), 3.56–3.64 (m, 1H), 6.98 (t, *J* = 8.6 Hz, 2H), 7.13 (t, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.6 Hz, 2H), 7.96 (td, *J* = 2.7, 10.2 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 21.8, 29.4, 29.6, 44.0, 45.4, 52.0, 53.1, 58.9, 62.0, 66.1, 69.7, 115.0, 115.2, 115.8, 116.0, 130.6, 130.7, 130.9, 131.0, 201.0. The optical rotation of (–)-14a was [*α*]_D²⁰ = –6.9° (1.0 mg/mL in MeOH). HRMS (ESI) Calcd for C₂₄H₂₉F₂N₂O₂ [M+H]⁺ 415.2192, found: 415.2192. (–)-TZ3108 was converted to its oxalate salt according to reported procedure. (–)-TZ3108 oxalate, mp: 205 °C (decomposed).

5.2.3. (4-Fluorophenyl)(3'-hydroxy-1'-(4-methoxybenzyl)-[1,4'-bipiperidin]-4-yl)methanone, (+)-14b—After two resolution processes, 27 mg of (+)-14b (ee 97.4%) was obtained. ¹H NMR (300 MHz, CDCl₃): δ 1.56 (q, *J* = 13.0 Hz, 1H), 1.74 (d, *J* = 11.6 Hz, 2H), 1.83 – 1.89 (m, 4H), 1.97 (t, *J* = 12.9 Hz, 1H), 2.27 (q, *J* = 12.9 Hz, 2H), 2.67 – 2.75 (m, 2H), 2.96 (t, *J* = 12.0 Hz, 2H), 3.16 – 3.26 (m, 2H), 3.49 (s, 2H), 3.60 (t, *J* = 9.6 Hz, 1H), 3.80 (s, 3H), 6.84 (d, *J* = 8.4 Hz, 2H), 7.10 – 7.21 (m, 4H), 7.96 (t, *J* = 7.1 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 21.8, 29.5, 29.6, 44.0, 45.5, 52.0, 53.0, 55.4, 58.6, 62.2, 66.2, 69.8, 113.8, 115.8, 116.1, 130.2, 130.5, 131.0, 131.1, 159.0, 201.1. MP: 200 °C (decomposed, oxalate salt). The optical rotation of (+)-14b was [*α*]_D²⁰ = +17.1° (1.0 mg/mL in MeOH). HRMS (ESI) Calcd for C₂₅H₃₂FN₂O₃ [M+H]⁺ 427.2391, found: 427.2392.

5.2.4. (4-Fluorophenyl)(3'-hydroxy-1'-(4-methoxybenzyl)-[1,4'-bipiperidin]-4-yl)methanone, (-)-14b—After two resolution processes, 22 mg of (-)-14b (ee 95.2%) was obtained. ¹H NMR (300 MHz, CDCl₃): δ 1.54 (q, *J* = 12.6 Hz, 1H), 1.71 (d, *J* = 12.6 Hz, 2H), 1.82 – 1.89 (m, 4H), 1.96 (t, *J* = 11.6 Hz, 1H), 2.24 (q, *J* = 11.6 Hz, 2H), 2.70 – 2.74 (m, 2H), 2.95 (t, *J* = 13.0 Hz, 2H), 3.18 – 3.23 (m, 2H), 3.48 (s, 2H), 3.59 (t, *J* = 9.8 Hz, 1H), 3.79 (s, 3H), 6.84 (d, *J* = 8.4 Hz, 2H), 7.09 – 7.21 (m, 4H), 7.95 (t, *J* = 7.2 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 21.8, 29.5, 29.6, 44.0, 45.5, 52.0, 53.0, 55.4, 58.9, 62.2, 66.2, 69.8, 113.8, 115.8, 116.1, 130.2, 130.5, 130.9, 131.1, 159.0, 201.0. MP: 203 °C (decomposed, oxalate salt). The optical rotation of (-)-14b was [*α*]_D²⁰ = -16.7° (6.0 mg/mL in MeOH). HRMS (ESI) Calcd for C₂₅H₃₂FN₂O₃ [M+H]⁺ 427.2391, found: 427.2393.

5.2.5. (1'-Benzyl-3'-hydroxy-[1,4'-bipiperidin]-4-yl)(4-fluorophenyl)methanone, (+)-14c—After two resolution processes, 28 mg of (+)-14c (ee 95.8%) was obtained. ¹H NMR (300 MHz, CDCl₃): δ 1.56 (q, *J* = 12.3 Hz, 1H), 1.70 (d, *J* = 11.7 Hz, 2H), 1.87 (d, *J* = 9.0 Hz, 4H), 1.99 (t, *J* = 11.5 Hz, 1H), 2.25 (q, *J* = 12.0 Hz, 2H), 2.67 – 2.75 (m, 2H), 2.97 (t, *J* = 12.6 Hz, 2H), 3.16 – 3.25 (m, 2H), 3.49 (s, 2H), 3.48 – 3.65 (m, 1H), 7.13 (t, *J* = 8.5 Hz, 2H), 7.21 – 7.34 (m, 5H), 7.96 (d, *J* = 7.5 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 21.8, 29.5, 29.6, 44.1, 45.5, 52.1, 53.2, 59.1, 62.9, 66.2, 69.8, 115.8, 116.1, 127.3, 128.4, 129.3, 131.0, 131.1, 138.3, 201.1. MP: 216 °C (decomposed, oxalate salt). The optical rotation of (+)-14c was [*α*]_D²⁰ = +45.0° (1.3 mg/mL in MeOH). HRMS (ESI) Calcd for C₂₄H₃₀FN₂O₂ [M+H]⁺ 397.2286, found: 397.2285.

5.2.6. (1'-Benzyl-3'-hydroxy-[1,4'-bipiperidin]-4-yl)(4-fluorophenyl)methanone, (-)-14c—After two resolution processes, 25 mg of (-)-14c (ee 95.5%) was obtained. ¹H NMR (300 MHz, CDCl₃): δ 1.55 (q, *J* = 12.1 Hz, 1H), 1.72 (d, *J* = 12.0 Hz, 2H), 1.85 – 1.92 (m, 4H), 1.97 (t, *J* = 11.5 Hz, 1H), 2.25 (q, *J* = 12.6 Hz, 2H), 2.67 – 2.76 (m, 2H), 2.96 (t, *J* = 11.3 Hz, 2H), 3.16 – 3.25 (m, 2H), 3.49 (s, 2H), 3.48 – 3.64 (m, 1H), 7.13 (t, *J* = 8.5 Hz, 2H), 7.22 – 7.33 (m, 5H), 7.96 (d, *J* = 7.5 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 21.8, 29.5, 29.6, 44.0, 45.5, 52.1, 53.2, 59.1, 62.9, 66.2, 69.8, 115.8, 116.1, 127.3, 128.4, 129.3, 131.0, 131.1, 138.3, 201.1. MP: 220 °C (decomposed, oxalate salt). The optical rotation of (-)-14c was [*α*]_D²⁰ = -46.7° (0.40 mg/mL in MeOH). HRMS (ESI) Calcd for C₂₄H₃₀FN₂O₂ [M+H]⁺ 397.2286, found: 397.2286.

5.2.7. HPLC resolution of compound (±)-(4-fluorophenyl)(3'-hydroxy-1'-(pyridin-3-ylmethyl)-[1,4'-bipiperidin]-4-yl)-methanone, 14d—HPLC condition: Chiralcel OD column (250 × 10 mm, 5:94.8:0.2 2-propanol/hexane/Et₃N, 5.0 mL/min). 20.0 mg of (+)-**14d** (*ee* > 99%) and 22.0 mg of (–)-**14d** (*ee* > 99%) were obtained.

(+)-**14d**: ¹H NMR (300 MHz, CDCl₃): δ 1.55 (q, *J* = 12.3 Hz, 1H), 1.71 – 1.79 (m, 2H), 1.87 – 1.96 (m, 4H), 2.03 (t, *J* = 11.4 Hz, 1H), 2.22 (q, *J* = 11.9 Hz, 2H), 2.68 – 2.76 (m, 2H), 2.96 (t, *J* = 13.8 Hz, 2H), 3.17 – 3.27 (m, 2H), 3.55 (s, 2H), 3.59 – 3.64 (m, 1H), 7.10 – 7.17 (m, 2H), 7.23 – 7.28 (m, 1H), 7.63 – 7.66 (m, 1H), 7.94 – 7.99 (m, 2H), 8.50 – 8.53 (m, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 21.6, 29.2, 29.4, 43.8, 46.1, 51.8, 53.0, 58.9, 59.8, 65.9, 69.4, 115.6, 115.9, 123.2, 130.8, 132.4, 133.6, 136.5, 148.6, 150.3, 200.8. MP: 163 °C (decomposed, oxalate salt). The optical rotation of (+)-**14d** was [*α*]_D²⁰ = +10.6° (0.72 mg/mL in MeOH). HRMS (ESI) Calcd for C₂₃H₂₉FN₃O₂ [M+H]⁺ 398.2238, found: 398.2234.

(–)-**14d**: ¹H NMR (300 MHz, CDCl₃): δ 1.57 (q, *J* = 12.3 Hz, 1H), 1.72 – 1.79 (m, 2H), 1.87 – 1.96 (m, 4H), 2.03 (t, *J* = 11.7 Hz, 1H), 2.27 (q, *J* = 11.0 Hz, 2H), 2.72 – 2.76 (m, 2H), 2.96 (t, *J* = 9.3 Hz, 2H), 3.17 – 3.27 (m, 2H), 3.55 (s, 2H), 3.58 – 3.64 (m, 1H), 7.11 – 7.16 (m, 2H), 7.23 – 7.29 (m, 1H), 7.63 – 7.66 (m, 1H), 7.95 – 7.99 (m, 2H), 8.50 – 8.53 (m, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 21.8, 29.4, 29.5, 43.9, 45.5, 52.0, 53.1, 59.1, 60.0, 66.1, 69.6, 115.8, 116.1, 123.4, 131.0, 132.6, 133.8, 136.7, 148.8, 150.5, 201.0. MP: 167 °C (decomposed, oxalate salt). The optical rotation of (–)-**14d** was [*α*]_D²⁰ = –10.6° (1.5 mg/mL in MeOH). HRMS (ESI) Calcd for C₂₃H₂₉FN₃O₂ [M+H]⁺ 398.2238, found: 398.2235.

5.2.8. (1'-(4-Fluorobenzyl)-3'-hydroxy-[1,4'-bipiperidin]-4-yl)(4-methoxyphenyl)methanone, (+)-14e—After two resolution processes, 21 mg of (+)-**14e** (*ee* > 99%) was obtained. ¹H NMR (300 MHz, CDCl₃): δ 1.59 (q, *J* = 13.9 Hz, 1H), 1.73 (d, *J* = 11.1 Hz, 2H), 1.83 – 1.90 (m, 4H), 1.98 (t, *J* = 11.5 Hz, 1H), 2.25 (q, *J* = 11.7 Hz, 2H), 2.67 – 2.75 (m, 2H), 2.94 (t, *J* = 13.5 Hz, 2H), 3.16 – 3.26 (m, 2H), 3.49 (s, 2H), 3.55 – 3.63 (m, 2H), 3.85 (s, 3H), 6.92 – 7.01 (m, 4H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.92 (d, *J* = 8.7 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 21.7, 29.5, 29.7, 43.6, 45.4, 52.1, 53.1, 55.5, 58.9, 61.9, 66.1, 69.7, 113.9, 114.9, 115.2, 129.1, 130.6, 130.7, 134.0, 163.5, 201.1. MP: 213 °C (decomposed, oxalate salt). The optical rotation of (+)-**14e** was [*α*]_D²⁰ = +14.3° (1.9 mg/mL in MeOH). HRMS (ESI) Calcd for C₂₅H₃₂FN₂O₃ [M+H]⁺ 427.2391, found: 427.2391.

5.2.9. (1'-(4-Fluorobenzyl)-3'-hydroxy-[1,4'-bipiperidin]-4-yl)(4-fluorophenyl)methanone, (–)-14e—After two resolution processes, 22 mg of (–)-**14e** (*ee* 96.0%) was obtained. ¹H NMR (300 MHz, CDCl₃): δ 1.56 (q, *J* = 12.0 Hz, 1H), 1.73 (d, *J* = 11.4 Hz, 2H), 1.78 – 1.90 (m, 4H), 1.98 (t, *J* = 11.7 Hz, 1H), 2.24 (q, *J* = 11.7 Hz, 2H), 2.71 – 2.75 (m, 2H), 2.95 (t, *J* = 13.5 Hz, 2H), 3.16 – 3.21 (m, 2H), 3.50 (s, 2H), 3.55 – 3.63 (m, 2H), 3.86 (s, 3H), 6.92 – 7.01 (m, 4H), 7.25 (d, *J* = 7.5 Hz, 2H), 7.92 (d, *J* = 9.0 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 21.8, 29.6, 29.8, 43.7, 45.5, 52.2, 53.2, 55.6, 59.0, 62.0, 66.2, 69.8, 114.0, 115.0, 115.3, 129.2, 130.7, 130.8, 134.1, 163.6, 201.2. MP: 215 °C (decomposed, oxalate salt). The optical rotation of (–)-**14e** was [*α*]_D²⁰ = –13.2° (6.0 mg/mL in MeOH). HRMS (ESI) Calcd for C₂₅H₃₂FN₂O₃ [M+H]⁺ 427.2391, found: 427.2394.

5.2.10. HPLC resolution of compound ($\pm$)-3'-hydroxy-1'-(4-methoxybenzyl)-[1,4'-bipiperidin]-4-yl(4-methoxyphenyl)-methanone, **14f**—HPLC condition:

Chiralcel OD column (250 $\times$ 10 mm, 5:94.8:0.2 2-propanol/hexane/Et₃N, 5.0 mL/min). 20.0 mg of (+)-**14f** (*ee* > 99%) and 21.0 mg of (–)-**14f** (*ee* > 99%) were obtained.

(+)-**14f**: ¹H NMR (300 MHz, CDCl₃): δ 1.59 (q, *J* = 13.6 Hz, 1H), 1.71 – 1.97 (m, 7H), 2.70 – 2.75 (m, 4H), 2.96 (t, *J* = 11.4 Hz, 2H), 3.20 – 3.24 (m, 2H), 3.49 (s, 2H), 3.60 (dt, *J* = 3.9, 9.6 Hz, 1H), 3.80 (s, 3H), 3.87 (s, 3H), 6.85 (d, *J* = 8.4 Hz, 2H), 6.94 (d, *J* = 9.0 Hz, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.92 (d, *J* = 9.0 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 21.8, 29.6, 29.8, 43.8, 45.6, 52.2, 53.0, 55.5, 55.7, 59.0, 62.3, 66.2, 69.8, 113.8, 114.0, 129.3, 130.2, 130.5, 130.7, 159.0, 163.6, 201.2. MP: 222 °C (decomposed, oxalate salt). The optical rotation of (+)-**14f** was $[\alpha]_D^{20} = +20.3^\circ$ (0.59 mg/mL in MeOH). HRMS (ESI) Calcd for C₂₆H₃₅N₂O₄ [M+H]⁺ 439.2591, found: 439.2589.

(–)-**14f**: ¹H NMR (300 MHz, CDCl₃): δ 1.54 (q, *J* = 13.6 Hz, 1H), 1.71 – 2.00 (m, 7H), 2.24 (q, *J* = 11.3 Hz, 2H), 2.70 – 2.74 (m, 2H), 2.96 (t, *J* = 10.8 Hz, 2H), 3.20 – 3.22 (m, 2H), 3.49 (s, 2H), 3.59 (dt, *J* = 4.2, 9.3 Hz, 1H), 3.80 (s, 3H), 3.87 (s, 3H), 6.84 (d, *J* = 8.1 Hz, 2H), 6.94 (d, *J* = 8.7 Hz, 2H), 7.20 (d, *J* = 8.1 Hz, 2H), 7.92 (d, *J* = 8.7 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 21.8, 29.7, 29.8, 43.8, 45.6, 52.2, 53.1, 55.5, 55.7, 58.9, 62.3, 66.3, 69.8, 113.8, 114.0, 129.3, 130.3, 130.6, 130.7, 159.0, 163.6, 201.2. MP: 226 °C (decomposed, oxalate salt). The optical rotation of (–)-**14f** was $[\alpha]_D^{20} = -21.3^\circ$ (0.89 mg/mL in MeOH). HRMS (ESI) Calcd for C₂₆H₃₅N₂O₄ [M+H]⁺ 439.2591, found: 439.2587.

5.2.11. HPLC resolution of compound ($\pm$)-(4-fluorophenyl)(3'-hydroxy-1'-(4-nitrobenzyl)-[1,4'-bipiperidin]-4-yl)methanone, **TZ3138**—Enantiomers of ($\pm$)-**TZ3138** (132 mg, 0.29 mmol) were separated by HPLC using a Chiralcel OD column (10 mm $\times$ 250 mm) consisting of mobile phase 2-propanol/hexane (1/4, v/v), flow rate of 4.0 mL/min, and UV wavelength at 254 nm. Under these conditions, 50 mg of (–)-**TZ3138** with retention time at 44 – 54 min, and 46 mg of (+)-**TZ3138** with retention time at 56 – 68 min were obtained. The enantiomeric excess of each enantiomer were determined by analytical Chiralcel OD HPLC (conditions: Chiral OD column, 4.6 mm $\times$ 250 mm, 2-propanol/hexane (1/4, v/v), flow rate 1 mL/min, detection wavelength 254 nm). The spectroscopic data were identical with the reported racemic precursor.²⁷

(+)-**TZ3138**: ¹H NMR (400 MHz, CDCl₃): δ 1.46 – 1.57 (m, 1H), 1.65 – 1.78 (m, 2H), 1.81 – 1.95 (m, 5H), 2.15 – 2.26 (m, 2H), 2.67 – 2.73 (m, 2H), 2.84 – 2.95 (m, 2H), 3.11 – 3.21 (m, 2H), 3.44 (dd, *J* = 13.6 Hz, 18.0 Hz, 2H), 3.59 (dt, *J* = 5.2 Hz, 9.6 Hz, 1H), 6.92 (t, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 7.99 (d, *J* = 8.4 Hz, 2H), 8.24 (d, *J* = 9.2 Hz, 2H). MP: 119 °C. The optical rotation for (+)-**TZ3138**, $[\alpha]_D^{20} = 14.0^\circ$ (1.0 mg/mL in MeOH).

(–)-**TZ3138**: ¹H NMR (400 MHz, CDCl₃): δ 1.44 – 1.52 (m, 1H), 1.62 – 1.72 (m, 2H), 1.78 – 1.95 (m, 5H), 2.14 – 2.26 (m, 2H), 2.64 – 2.73 (m, 2H), 2.84 – 2.95 (m, 2H), 3.11 – 3.22 (m, 2H), 3.36 (br, 1H), 3.44 (dd, *J* = 13.6 Hz, 18.0 Hz, 2H), 3.52 (dt, *J* = 4.8 Hz, 10.0 Hz, 1H), 6.92 (t, *J* = 8.4 Hz, 2H), 7.18 (d, *J* = 6.0 Hz, 2H), 7.99 (d, *J* = 8.4 Hz, 2H), 8.25 (d, *J* =

8.8 Hz, 2H). MP: 119 °C. The optical rotation for (–)-**TZ3138**, $[\alpha]_D^{20} = -17.0^\circ$ (1.0 mg/mL in MeOH).

5.3. *In vitro* competitive binding assay

The competitive binding assays towards σ_1 , σ_2 , VACHT were performed according to the published protocols.^{27, 36, 37} In brief, the σ_1 receptor binding assays were conducted in 96-well plates with guinea pig brain membrane homogenates as the receptor source, and (+)-[³H]pentazocine (PerkinElmer, Boston, MA) as the competitive radioligand. 10 μ M nonradiolabeled haloperidol was added to the plates to determine the nonspecific binding. For selectivity measurement of **TZ3108** and its enantiomers, the σ_2 receptor binding assays were conducted with rat liver membrane homogenates as the receptor source, and [³H]DTG as the competitive radioligand. 1 μ M (+)-pentazocine was added to block σ_1 binding sites. 10 μ M nonradiolabeled haloperidol was added to measure the nonspecific binding. Nonlinear regression analysis was used to determine the IC₅₀ concentration. *In vitro* VACHT binding assays of racemic **TZ3108** and its enantiomers were conducted on human VACHT that was expressed in PC12 (A123.7) cells. (–)-[³H]vesamicol was used as the competitive radioligand. Nonradiolabeled (–)-vesamicol ($K_i = 15$ nM) was used as an external standard. The K_i values were calculated by regression analysis. The assay for each compound was independently assayed at least twice.

5.4. Radiochemistry

A bolus of 7 – 8 GBq [¹⁸F]/fluoride in 1.0 mL syringe was added to a reaction tube (13 × 100 mm, 8 mL) containing Kryptofix 2.2.2 (6 – 8 mg). The syringe was rinsed with 2 × 0.4 mL ethanol. The resulting solution was evaporated under gentle nitrogen flow at 110 °C in an oil bath. Acetonitrile (1.0 mL) was added to the residue and was again dried under nitrogen flow and heating. The drying procedure was repeated twice. 2 – 3 mg of the corresponding precursor (–)-**TZ3138** was dissolved in DMSO (300 μ L) under vortex, and 150 μ L of the solution was transferred into the reaction vessel containing [¹⁸F]fluoride/Kryptofix 2.2.2/K₂CO₃. The reaction tube was capped and the reaction mixture was briefly mixed and then subjected to heating in an oil bath for 10 min at 125 °C. The reaction vial was removed from the oil bath and the remaining 150 μ L reaction solution was added. The resulting mixture was briefly mixed and heated at 125 °C for another 10 min. The reaction mixture was quenched with 3.0 mL of HPLC mobile phase (acetonitrile/0.1 M ammonium formate buffer, 1/4, v/v, pH ~4.5) and passed through an alumina Neutral Sep-Pak Plus cartridge. The crude product was then loaded onto a Phenomenex Luna C18 semi-preparative HPLC column (250 mm × 9.6 mm) with a UV detector set at 254 nm. The HPLC system used a 5 mL injection loop. The isocratic mobile phase consisted of 20% acetonitrile in 80% 0.1 M ammonium formate buffer (pH ~4.5) and a flow rate at 4.0 mL/min. The HPLC fraction of the product was collected into a vial with ~50 mL water and the diluted solution was passed through a C-18 Sep-Pak Plus cartridge to trap the product on the Sep-Pak. Then the Sep-Pak was rinsed with another 20 mL Mini-Q water. The trapped activity was eluted with USP grade ethanol (0.6 mL) followed by 5.4 mL of 0.9% saline. After sterile filtration into a glass vial, the final product was ready for quality control (QC) analysis and animal studies. The retention time for the product was 39 – 42 min. The precursor retention time was 43 – 47 min. An aliquot of sample was assayed by an analytical

HPLC system (Phenomenex Prodigy C18 column, 250 × 4.6 mm) using a detection wavelength at 254 nm and a mobile phase consisting of acetonitrile/0.1 M ammonium formate buffer (38/62, v/v, pH 4.5). Using these conditions the retention time for (–)-[¹⁸F]TZ3108 was approximately 5.4 min at a flow rate of 1 mL/min. The sample was authenticated by co-injecting with the nonradiolabeled standard reference solution. The labeling yield was 19 ± 2% with a specific activity of 58 – 84 GBq/μmol (decay corrected to the end of synthesis). The radiochemical purity was > 99% and the chemical purity was > 95%. The entire procedure took about 70 min.

5.5. Biodistribution study in SD rats

All the experiments conducted in rodents and NHPs were in compliance with the Guidelines for the Care and Use of Research Animals under protocols approved by Animal Studies Committee at the Washington University School of Medicine. For the biodistribution studies, the formulation of (–)-[¹⁸F]TZ3108 (2.1 – 2.4 MBq in 10% EtOH, 90% saline, 120 μL) was injected via the tail vein into adult male SD rats under isoflurane/oxygen anesthesia. The rats were euthanized under anesthesia at 5, 30, 60, and 120 min p.i. (n = 3 for each time point). The whole brain was immediately harvested and dissected into regions including cortex, striatum, thalamus, hippocampus, and cerebellum; the rest of the brain was also collected to determine the total brain uptake. Peripheral organs and tissues including blood, heart, lung, muscle, fat, pancreas, spleen, kidney, liver, and bone were also collected; all samples were counted in an automated Beckman Gamma 8000 well counter with a standard dilution of (–)-[¹⁸F]TZ3108. Counts were decay-corrected and the %ID/g was calculated.

5.6. MicroPET/CT scans in cynomolgus monkey brain

Brain microPET imaging of (–)-[¹⁸F]TZ3108 was performed on a microPET Focus 220 scanner (Concorde/CTI/Siemens Microsystems, Knoxville, TN), and imaging data processing was conducted according to our published procedures.²⁸ In this study the baseline scan and the blocking scan were done with same NHP subject. The subject was intubated and anesthetized with ~2% isoflurane in oxygen upon arrival at the scanner. A percutaneous catheter was placed in the femoral vein for injection of (–)-[¹⁸F]TZ3108 or blocking agents Yun122 and SA4503. Anesthesia was maintained at 0.75–2.0% during the whole imaging session and core temperature was maintained at 37 °C with a water blanket; vital signs were monitored every 5 min. A 10 min transmission scan was conducted to confirm positioning of the NHP brain within the scanner, followed by a 30 min transmission scan for attenuation correction. Thereafter, a 2 hour dynamic (3 × 1-min, 4 × 2-min, 3 × 3-min, and 20 × 5-min frames) emission scan was performed after i.v. injection of 0.33 – 0.45 GBq of (–)-[¹⁸F]TZ3108 in 10% ethanol saline solution. For blocking studies, a known σ₁ compound Yun122 (1 mg/kg) or SA4503 (1.5 mg/kg) was injected i.v. 5 min prior to the tracer injection; data acquisition was the same as the baseline scan. PET emission data were corrected by related attenuation and reconstructed through filtered back projection. The MRI (MPRAGE) was co-registered to the baseline PET image. All volume of interest (VOI) were drawn on the MPRAGE image using Vidi or Analyze program (AnalyzeDirect Inc., Overland Park, KS). Three-dimensional VOIs were transformed to the PET space and then overlaid on all reconstructed PET images to obtain TACs. Activity measures were standardized to the NHP body weight, and injected dose of radioactivity yielded SUV.

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Abbreviations

calcd	calculated
CH_2Cl_2	dichloromethane
CNS	central nervous system
DMSO	dimethyl sulfoxide
$[\text{}^3\text{H}]\text{DTG}$	$[\text{}^3\text{H}]\text{ditolylguanidine}$
DTTA	di- <i>p</i> -toluoyl tartaric acid
EBP	emopamil binding protein
<i>ee</i>	enantiomeric excess
EOB	end of bombardment
EtOH	ethanol
$[\text{}^{18}\text{F}]\text{FTC146}$	^{18}F -6-(3-fluoropropyl)-3-(2-(azepan-1-yl)ethyl)benzo[d]thiazol-2(3 <i>H</i>)-one
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
IC_{50}	half maximum inhibitory constant
K_2CO_3	potassium carbonate
MeOH	methanol
MRI	magnetic resonance imaging
NaOH	sodium hydroxide
Na_2SO_4	sodium sulfate
NHPs	nonhuman primates

PDE01A	phosphodiesterase 10A
PET	positron emission tomography
p.i	post-injection
QC	quality control
ROIs	Regions of interest
[¹¹C]SA4503	1-(4-methoxy-3-(methoxy- ¹¹ C)phenethyl- ¹¹ C)-4-(3-phenylpropyl)piperazine
SD	Sprague Dawley
SUV	standardized uptake value
VAcHT	Vesicular Acetylcholine Transporter
VOI	volume of interest
Yun122	N-(4-benzylcyclohexyl)-2-(2-fluorophenyl)acetamide

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Figure 1.
PET radiotracers and lead prezamicol pharmacophore of sigma 1 ligands

Figure 2.

Analytical chromatograph of the enantiomers. A) HPLC chromatography of (-)-**TZ3138** (Chiralcel OD column, 250×4.6 mm, mobile phase: 80% hexane and 20% 2-propanol flow rate 1 mL/min, UV wavelength of 254 nm); B) HPLC chromatography of (-)-**TZ3108**.

Figure 3.

PET studies of (-)-[^{18}F]TZ3108 in the brain of a nonhuman primate. A) NHP brain PET/MR images from baseline dynamic scans showed high uptake of (-)-[^{18}F]TZ3108 in the brain. Regions of interest (ROI) were outlined manually on each MRI slice and re-grouped as: 1. Frontal cortex; 2. Striatum; 3. Thalamus; 4. Hippocampus; 5. Cerebellum. Color scale indicates SUV units. B) Brain tissue time activity curve (TAC) of (-)-[^{18}F]TZ3108 from the baseline scan.

Figure 4. Standardized uptake value of $(-)-[^{18}\text{F}]\text{TZ3108}$ in NHP brain under baseline condition and two pretreatment conditions. The σ_1 blocking agent Yun122 (1.0 mg/kg) or SA4503 (1.5 mg/kg) was administrated 5 min prior to the injection of $(-)-[^{18}\text{F}]\text{TZ3108}$. The data represent the summed SUV of 80 – 100 min scan.

Scheme 1.
Radiosynthesis of $(-)-[^{18}\text{F}]\text{TZ3108}$

Table 1

Enantiomeric resolution of **14a–14f** and their σ_1 , σ_2 , VACHT binding affinities^a

No.	Resolving method	K_i , nM										$\log P^{ab}$					
		(±)-racemic compound					(+) isomer						(-) isomer				
		σ_1^c	σ_2^d	VAcHT ^e	σ_1	σ_2	VAcHT	σ_1	σ_2	VAcHT	σ_1		σ_2	VAcHT			
14a	crystallization	0.48 ± 0.14	1740 ± 280	1360 ± 295	0.14 ± 0.02	2390 ± 340	1090 ± 200	1.8 ± 0.4	6960 ± 810	980 ± 87	2.83						
14b	crystallization	2.8 ± 0.5	5520 ± 1350	3310 ± 910	0.73 ± 0.08	> 10,000	6800 ± 2630	4.1 ± 0.6	> 10,000	2880 ± 860	2.61						
14c	crystallization	1.2 ± 0.2	2300 ± 250	400 ± 42	0.85 ± 0.14	4190 ± 410	370 ± 110	2.2 ± 0.4	3560 ± 180	130 ± 14	2.73						
14d	HPLC	23.0 ± 2.0	4210 ± 110	2030 ± 380	12.0 ± 3.0	7190 ± 930	6110 ± 1170	57 ± 11	> 10,000	740 ± 120	1.48						
14e	crystallization	2.5 ± 0.3	2790 ± 720	294 ± 16	0.82 ± 0.09	6370 ± 530	110 ± 26	30 ± 2	3910 ± 200	170 ± 57	2.82						
14f	HPLC	26.0 ± 1.0	5160 ± 200	> 10,000	23.0 ± 3.0	> 10,000	5490 ± 1780	104 ± 5	6940 ± 960	6330 ± 1770	2.61						

^a K_i values (mean ± SEM) were determined in at least three experiments.

^bCalculated value at pH 7.4 with ACD/ILab, version 7.0 (Advanced Chemistry Development, Inc., Canada).

^cThe σ_1 binding assay used membrane preparations of guinea pig brain.

^dThe σ_2 binding assay used homogenates of rat liver.

^eThe VACHT binding assay used expressed human VACHT.

Table 2

Biodistribution and regional brain biodistribution of (–)-[¹⁸F]TZ3108 in male Sprague Dawley rats (mean ± SD, %ID/g, n = 3)

Organs	5 min	30 min	60 min	120 min
Blood	0.051 ± 0.017	0.025 ± 0.001	0.020 ± 0.008	0.013 ± 0.003
Heart	1.39 ± 0.24	0.42 ± 0.06	0.25 ± 0.03	0.20 ± 0.02
Lung	12.29 ± 0.86	3.38 ± 0.33	1.88 ± 0.38	1.34 ± 0.18
Muscle	0.089 ± 0.031	0.11 ± 0.03	0.084 ± 0.015	0.080 ± 0.004
Fat	0.021 ± 0.004	0.075 ± 0.014	0.085 ± 0.012	0.097 ± 0.031
Pancreas	1.74 ± 0.69	1.81 ± 1.09	1.67 ± 0.70	1.60 ± 0.50
Spleen	2.52 ± 0.51	2.83 ± 0.64	2.71 ± 0.76	1.88 ± 0.50
Kidney	4.75 ± 0.39	2.76 ± 0.09	2.12 ± 0.03	1.64 ± 0.21
Liver	1.62 ± 0.42	2.76 ± 0.40	2.84 ± 1.49	2.81 ± 0.87
Bone	0.32 ± 0.01	0.35 ± 0.01	0.37 ± 0.04	0.32 ± 0.03
Regional brain biodistribution				
Cortex	1.652 ± 0.067	1.332 ± 0.124	0.960 ± 0.152	0.877 ± 0.189
Striatum	1.021 ± 0.057	0.890 ± 0.087	0.735 ± 0.111	0.684 ± 0.107
Thalamus	1.124 ± 0.101	0.954 ± 0.153	0.893 ± 0.135	0.810 ± 0.149
Hippocampus	1.186 ± 0.133	1.006 ± 0.156	0.805 ± 0.100	0.790 ± 0.108
Cerebellum	1.370 ± 0.069	1.002 ± 0.159	0.880 ± 0.067	0.796 ± 0.137
Total brain	1.285 ± 0.062	1.058 ± 0.133	0.864 ± 0.092	0.802 ± 0.129