Discovery and development of brain-penetrant 18F-labeled radioligands for neuroimaging of the sigma-2 receptors

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Abstract We have discovered and synthesized a series of indole-based derivatives as novel sigma-2 (σ2) receptor ligands. Two ligands with high σ2 receptor affinity and subtype selectivity were then radiolabeled with F-18 in good radiochemical yields and purities, and evaluated in rodents. In biodistribution studies in male ICR mice, radioligand [18F]9, or 1-(4-(5,6-dimethoxyisoindolin-2-yl)butyl)-4-(2-[18F]fluoroethoxy)-1H-indole, was found to display high brain uptake and high brain-to-blood ratio. Pretreatment of animals with the selective σ2 receptor ligand CM398 led to significant reductions in both brain uptake (29%–54%) and brain-to-blood ratio (60%–88%) of the radioligand in a dose-dependent manner, indicating high and saturable specific binding of [18F]9 to σ2 receptors in the brain. Further, ex vivo autoradiography in male ICR mice demonstrated regionally heterogeneous specific binding of [18F]9 in the brain that is consistent with the distribution pattern of σ2 receptors. Dynamic positron emission tomography imaging confirmed regionally distinct distribution and high levels of specific binding for [18F]9 in...
1. Introduction

Malfunctions in cholesterol homeostasis and lipid metabolism are involved in various human diseases. Recently, the sigma-2 (σ2) receptor has been identified as a transmembrane protein 97 (TMEM97), which is also named meningioma-associated protein (MAC30) and suggested to be a cholesterol-regulating gene. Accordingly, much attention has been paid to the mechanism and role of the σ2 receptor/TMEM97 in regulating cholesterol homeostasis and its implications in diseases. The σ2 receptor/TMEM97 was shown to have approximately 10-fold higher expression in proliferating than quiescent tumors, indicating the σ2 receptor/TMEM97 as a biomarker for the proliferative status of solid tumors. TMEM97, progesterone receptor membrane component 1 (PGRMC1) and low-density lipoprotein receptor (LDLR) complex, leading to decreased uptake of Aβ2 receptor/TMEM97 in 66P and 66Q cells. Moreover, the TMEM97/PGRMC1/LDLR complex is a binding site for monomeric and oligomeric amyloid β-peptide (1–42) (Aβ1–42). Inhibition of one of these proteins results in the disruption of the TMEM97/PGRMC1/LDLR complex, leading to decreased uptake of Aβ1–42 in primary neurons. Hence, the σ2 receptor/TMEM97 is also considered a therapeutic target for Alzheimer’s disease (AD). For example, CT1812, a σ2 receptor antagonist, is reported to prevent the binding of Aβ oligomers to neuronal receptors and thus holds potential as a novel drug for the treatment of AD.

The availability of a radioligand for neuroimaging of the σ2 receptor/TMEM97 in the human brain will make it possible to investigate this target in AD progression, and to elucidate the treatment mechanism of CT1812 in clinical trials.

During the last decades, efforts in the development of σ2 receptor radioligands have been largely directed toward in vivo imaging of tumors, where upregulation of the σ2 receptor is found. Currently, N-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-2-(2-fluoroethyl)-5-methylbenzamide ([18F]ISO-1) is the only σ2 receptor radiotracer used in humans for tumor imaging. However, it is not suitable to investigate σ2 receptors in AD due to its low brain uptake. With the aim to develop radioligands with appropriate affinity for the σ2 receptor, high selectivity, high uptake, favorable kinetics and high specific binding in the brain, we chose compound 1 with nanomolar affinity and favorable selectivity for σ2 receptor as the lead compound and introduced an methoxy or fluoroethoxy group at the different positions of the indole ring to enable the incorporation of [11C] or [18F] for PET imaging (Fig. 1). Previous structure–activity relationship studies showed that ligands with a four-carbon chain 

the rat brain, along with appropriate tissue kinetics. Taken together, results from our current study indicated the novel radioligand [18F]9 as the first highly specific and promising imaging agent for σ2 receptors in the brain.

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2.3. Radiochemistry

Synthesis of the radiolabeling precursors and radiosynthesis of radioligands [\(^{18}\)F]9 and [\(^{18}\)F]10 are depicted in Scheme 2. Intermediates 20 and 21 were obtained by reaction of 4-(benzylxy)-1H-indole (18) or 5-(benzylxy)-1H-indole (19) with 1,4-dibromobutane under basic conditions. N-Alkylation of 5,6-dimethoxyisoindoline with 20 and 21 provided 22 and 23, respectively. Debenzylation in the presence of H\(_2\) and 10% Pd on activated carbon as catalyst provided compounds 24 and 25. Further reaction with ethane-1,2-diyl bis(4-methylbenzenesulfonate) afforded compounds 26 and 27 as radiolabeling precursors.

Different from the synthetic methods previously described for [\(^{18}\)F]8 and [\(^{18}\)F]11, we employed for the radiosynthesis of [\(^{18}\)F]9 and [\(^{18}\)F]10 an efficient one-step S\(_{N}\)2 reaction with [\(^{18}\)F]fluoride as reported in the literature\(^{32}\), with retention times of 16.06 and 16.16 min on the chromatograms from semipreparative radio-high performance liquid chromatography (radio-HPLC, Supporting Information Fig. S1), radiochemical yields (RCYs) of 36%–50% (n = 10, decay-corrected) and 20%–29% (n = 5, decay-corrected), molar activity of 29–151 GBq/\(\mu\)mol (n = 10) and 55–72 GBq/\(\mu\)mol (n = 5), respectively, for [\(^{18}\)F]9 and [\(^{18}\)F]10, and radiochemical purity (RCP) of >99%. The total synthesis time was about 60 min. The RCYs for [\(^{18}\)F]9 and [\(^{18}\)F]10 from the one-step radiosynthesis were considerably higher than those for [\(^{18}\)F]8 (10%–15%) and [\(^{18}\)F]11 (16%–21%) prepared from a two-step procedure reported previously\(^{29}\).

Identity of the radioligand [\(^{18}\)F]9 or [\(^{18}\)F]10 was confirmed by co-injection with the corresponding reference compound 9 or 10 into an analytical HPLC system. The chromatograms are shown in Supporting Information Fig. S2, indicating co-elution of the labeled and unlabeled compounds. The retention times were 8.53 and 8.61 min for 9 and [\(^{18}\)F]9, and 8.19 and 8.31 min for 10 and [\(^{18}\)F]10, respectively, with the slight differences in retention times accounted for the distance between the UV and gamma detectors of the HPLC system.

2.4. In vitro and in vivo assessments

2.4.1. In vitro stability

Radioligands [\(^{18}\)F]9 and [\(^{18}\)F]10 were incubated in saline at ambient temperature or in mouse serum at 37 °C for 2 h to determine their stability in vitro. The RCP remained at >99% as shown in Supporting Information Fig. S3, suggesting high stability of [\(^{18}\)F]9 and [\(^{18}\)F]10 in vitro.

2.4.2. Lipophilicity

Radioligands [\(^{18}\)F]9 and [\(^{18}\)F]10 were partitioned between 1-octanol and 0.05 mol/L potassium phosphate buffer (pH 7.4) to measure their log\(D_{o/w}\) which were 2.43 ± 0.01 (n = 3) and 2.29 ± 0.01 (n = 3), respectively, for [\(^{18}\)F]9 and [\(^{18}\)F]10, well within the optimal range for good blood–brain barrier (BBB) permeability\(^{33}\).

2.4.3. Biodistribution and blocking experiments in mice

Biodistribution experiments were performed in male ICR mice. The results are presented in Tables 2 and 3. Initial uptake in the brain, at 2 min post-injection (p.i.), was observed to be higher for

Table 1  In vitro binding properties of the indole-based ligands\(^a\).

| Compd. | \(K_i (\mu\text{M})\) | \(K_i (\mu\text{M})\) | \(K_i (\mu\text{M})\) |
|--------|----------------|----------------|----------------|
| 2      | 149 ± 67        | 6.30 ± 2.00    | 24             |
| 3      | 190 ± 97        | 8.91 ± 1.40    | 21             |
| 4      | 70 ± 14         | 9.46 ± 1.20\(^c\) | 7              |
| 5      | 259 ± 23        | 7.73 ± 4.49\(^c\) | 33             |
| 6      | 450 ± 89        | 4.40 ± 1.43\(^c\) | 102            |
| 7      | 357 ± 94        | 8.68 ± 1.99    | 41             |
| 8\(^b\) | 1698 ± 548      | 2.40 ± 0.58    | 708            |
| 9\(^b\) | 371 ± 105       | 1.79 ± 0.86    | 207            |
| 10     | 187 ± 1.41      | 3.27 ± 0.19    | 57             |
| 11     | 376 ± 351       | 2.63 ± 0.48    | 143            |

\(^a\)Values are the means ± standard deviation (SD) of at least two independent experiments, each done in triplicate.

\(^b\)From Ref. 29.

\(^c\)n = 2.

\(^d\)Reagents and conditions: (a) DMF, 1,4-dibromobutane, KOH, TBAF (1 mol/L in THF), r.t., 2 h, 52%–72%; (b) acetonitrile, triethylamine, K\(_2\)CO\(_3\), 5,6-dimethoxyisoindoline/6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, 90 °C, 5 h, 55%–77%.
[18F]9 (4.44 ± 0.59% ID/g) than [18F]10 (3.76 ± 0.28% ID/g). Ligand [18F]9 showed the highest uptake in the brain (5.04 ± 0.67% ID/g) at 30 min, indicating a slow clearance. On the other hand, [18F]10 showed relatively fast brain washout, with level of uptake at 120 min only 42% of that at 2 min. The blood level of [18F]9 was low with 0.48 ± 0.08% ID/g at 30 min and 0.42 ± 0.05% ID/g at 60 min p.i., which led to high brain-to-blood ratios of 10.6 at both time points. The highest brain-to-blood ratio (3.13) was seen at 15 min for [18F]10.

To verify the binding specificity of [18F]9 and [18F]10 to σ2 receptors in vivo, blocking experiments in male ICR mice were conducted by pre-administration (5 min prior to ~280 kBq of radiotracer) of 9 (0.1 mL, 3 μmol/kg), 10 (0.1 mL, 3 μmol/kg), and various concentrations of CM398 (0.1 mL; 3, 5, 10 or 20 μmol/kg), a selective σ2 receptor ligand. The blocking effects on [18F]9 and [18F]10 at 30 min p.i. are summarized in Fig. 2. For [18F]9, animals pre-treated with CM398 showed a dose-dependent reductions in brain uptake levels (29%–54%), and brain-to-blood ratios (60%–88%). Activity levels were also reduced in the heart (66%–77%), spleen (55%–65%), lungs (73%–82%) and kidneys (73%–76%). Similarly, self-blocking led to remarkable reductions of radioactivity concentrations in the above organs.

For [18F]10, pretreatment with compound 10 or CM398 led to slightly reduced activity uptake in the brain (8%–18%). The brain-to-blood ratio also decreased (37%–47%). In addition, radioactivity levels were reduced in the liver (30%–42%), spleen (47%–54%), and kidneys (31%–33%). Overall the blocking effect was smaller for [18F]10 than [18F]9, indicating lower levels of specific binding for [18F]10.

2.4.5. Ex vivo autoradiography in mice

Given the more favorable binding property observed for [18F]9 in biodistribution studies, ex vivo autoradiography study was conducted to investigate its levels of uptake and specific binding in mouse brain regions. The results are presented in Figs. 4–7. Radioactivity was widely and heterogeneously distributed in the mouse brain. High levels of accumulation were observed in the cortex, cerebellum, olfactory bulb, midbrain and lateral ventricle, similar to the results reported in the literature. Uptake level was moderate in the striatum, and low in the thalamus and hippocampus. The pattern of distribution in the brain for [18F]9 is clearly different from that we recently obtained with a σ1 receptor radioligand.

When the mice were injected with CM398 (5 μmol/kg) or compound 9 (3 μmol/kg) at 5 min before radiotracer injection and sacrificed at 30 min p.i. for autoradiography, levels of radiotracer accumulation were dramatically reduced in all brain regions (Figs. 4–7), with significant differences (P < 0.001) found in the cortex, hippocampus, midbrain, cerebellum, striatum, thalamus and olfactory bulb. The blocking effect averaged across brain regions was similar to that seen for the whole brain in the biodistribution study, reaffirming the high level of specific binding for [18F]9 in the brain.

2.4.6. Micro-PET/CT imaging in rats

Encouraged by the favorable properties of [18F]9 in mice, imaging experiments in Sprague–Dawley (SD) rats were performed on a
micro-postion emission tomography (PET)/computed tomography (CT) scanner to further investigate the pharmacokinetic and binding characteristics of [18F]9 in the brain. After administration of [18F]9, dynamic PET scan was acquired for 120 min. PET images are shown in Fig. 8. Presented in Fig. 9 are the whole brain and regional time–activity curves (TACs) from both the baseline and CM398 blocking scans. The highest levels of uptake were found in the cerebellum and cortex, followed by the hippocampus, midbrain, striatum and thalamus. Pretreatment with CM398 (10 μmol/kg) at 5 min prior to the injection of [18F]9 resulted in markedly decreased uptake in the above brain regions with faster washout. Radiotracer accumulation in the whole-brain was markedly decreased uptake in the above brain regions with faster washout. Radiotracer accumulation in the whole-brain was markedly decreased uptake in the above brain regions with faster washout. 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entered the brain well. Nonetheless, the level of specific binding was only moderate, and radioligand \(^{18}\text{F}\)ISO-1 (0.76% ID/g) \(^{27}\) with the 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline pharmacophore, demonstrated less favorable \emph{in vivo} kinetic and binding characteristics. In a continued effort to search for \(\sigma_2\) radioligands with improved kinetic and imaging properties, we further synthesized radioligands \(^{18}\text{F}\)ISO-9 and \(^{18}\text{F}\)ISO-10 with the \(^{18}\text{F}\)fluoroethoxy group at the 4- and 5-position of the indole ring, respectively, and the less lipophilic 5,6-dimethoxyisoindoline pharmacophore. As indicated by the results from biodistribution studies reported previously \(^{29}\), compared to \(^{18}\text{F}\)ISO-8, radiotracer \(^{18}\text{F}\)ISO-9, with the 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline pharmacophore exhibited lower initial brain uptake (3.29% ID/g at 2 min vs. 4.44% ID/g for \(^{18}\text{F}\)ISO-9) and lower brain-to-blood ratio (3.0 at 15 min vs. 7.2 for \(^{18}\text{F}\)ISO-9). Moreover, blocking with compound \(n\) led to only 24% reduction in brain uptake, suggesting low level of specific binding to \(\sigma_2\) receptors in the brain. So we focused on ligands with the 5,6-dimethoxyisoindoline pharmacophore to further investigate the effects of \(^{18}\text{F}\)fluoroethoxy at the different positions of the indole ring on brain kinetics and specific binding.

Listed in Table 4 for comparison are the \emph{in vitro} and \emph{in vivo} characteristics of radioligands \(^{18}\text{F}\)ISO-9, \(^{18}\text{F}\)ISO-10 and \(^{18}\text{F}\)ISO-11, with the \(^{18}\text{F}\)fluoroethoxy moiety at the 4-, 5-, or 6-position of the indole ring, respectively. Similar to \(^{18}\text{F}\)ISO-11, the two new radioligands \(^{18}\text{F}\)ISO-9 and \(^{18}\text{F}\)ISO-10 displayed the appropriate lipophilicity required for BBB permeability of neuroimaging agents, and good \emph{in vitro} stability. Both \emph{in vitro} binding affinity for \(\sigma_2\) receptor and \(\sigma_2/\sigma_1\) subtype selectivity followed the order of \(^{18}\text{F}\)ISO-9 > \(^{18}\text{F}\)ISO-11 > \(^{18}\text{F}\)ISO-10. Radioligands \(^{18}\text{F}\)ISO-9 and \(^{18}\text{F}\)ISO-11 displayed initial brain uptake at 2 min after injection (4.44% and 4.55% ID/g) higher than \(^{18}\text{F}\)ISO-10 (3.76% ID/g), which are all much higher than that of the established \(\sigma_2\) radioligand \(^{18}\text{F}\)ISO-1 (0.76% ID/g) \(^{27}\). Brain uptake at 30 min was similar for \(^{18}\text{F}\)ISO-10 and \(^{18}\text{F}\)ISO-11 (2.68% and 2.28% ID/g), but much higher for \(^{18}\text{F}\)ISO-9 (5.04% ID/g), which is consistent with its highest \(\sigma_2\) affinity \([K_s (\sigma_2) = 1.79 \pm 0.86 \text{ nmol/L}]\) in this series. Radioligand \(^{18}\text{F}\)ISO-9 also showed the highest brain-to-blood ratios, peaking at >10 between 30 and 60 min, compared with 3.0 and 5.0 for \(^{18}\text{F}\)ISO-10 and \(^{18}\text{F}\)ISO-11, respectively, at 15 min after injection. Further, in self-blocking or CM-398 pretreatment experiments, \(^{18}\text{F}\)ISO-9 displayed the most remarkable reduction of radioactivity levels in the brain, heart, spleen, lungs and kidneys, indicating that \(^{18}\text{F}\)ISO-9 in both the brain and blood. The differences in brain uptake and brain-to-blood ratio between control and cyclosporine A-treated groups were significant \((P < 0.05)\), indicating that \(^{18}\text{F}\)ISO-9 is a moderate P-gp substrate. Results from the same set of experiments for \(^{18}\text{F}\)ISO-10 and \(^{18}\text{F}\)ISO-11 also indicated that they might be substrates for P-gp.
Since $[^{18}F]9$ has the highest $\sigma_2$ specific binding in the brain in this series, ex vivo autoradiography experiments were conducted to further examine its distribution in brain regions. High levels of accumulation were found in the cortex, cerebellum and olfactory bulb, in line with results found with other $\sigma_2$ radioligands in the literature\textsuperscript{36,40}. On the other hand, higher levels of accumulation in the midbrain and lateral ventricle were also observed, with lower levels in the hippocampus and thalamus, which are clearly different from the regional expression pattern of $\sigma_1$ receptors in the brain\textsuperscript{32,37,41,42}, and thus establishing that radioligand $[^{18}F]9$ is evidently binding to the $\sigma_2$ receptors. These results verify that $\sigma_1$ and $\sigma_2$ receptors are distinctly and differently expressed in brain regions\textsuperscript{33}. Pretreatment of animals with CM398 and compound 9 led to significant reductions in radiotracer uptake in different brain areas, further indicating the highly specific nature of $[^{18}F]9$ binding to $\sigma_2$ receptors in the mouse brain and reaffirming the results from biodistribution studies.

Micro-PET/CT imaging was carried out in SD rats to further investigate the pharmacokinetic and binding properties of $[^{18}F]9$ in vivo. After intravenous injection, $[^{18}F]9$ entered the brain rapidly, then slowly washed out from different rat brain regions, consistent with the findings from biodistribution experiments in mice. Highest levels of uptake were observed in the cerebellum and cortex, followed by striatum, hippocampus, midbrain, and thalamus, in line with those found from ex vivo autoradiography study. Blocking with CM398 led to significant reductions in activity uptake to almost homogeneous levels in different brain regions, and much faster washout rates, demonstrating high specific binding of $[^{18}F]9$ to $\sigma_2$ receptors in the rat brain.

Metabolism study in mice indicated that $[^{18}F]9$ was the predominant radioactive species in the brain, with negligible amount of radio-metabolites, which is in good agreement with the high specific binding of $[^{18}F]9$ in the mouse brain. However, only about 10% of the radioactivity was attributed to $[^{18}F]9$ in the pancreas. As a result, there appeared to be little specific binding of $[^{18}F]9$ in

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Figure 4  Ex vivo autoradiograms of $[^{18}F]9$ in the mouse brain under baseline (upper row) and CM398 blocking (lower row) conditions: (A) Cortex; (B) Hippocampus; (C) Midbrain; (D) Cerebellum; (E) Lateral ventricle; (F) Striatum; (G) Thalamus; (H) Olfactory bulb.

Figure 5  Ex vivo autoradiography results of $[^{18}F]9$ in the mouse brain under baseline and CM398 blocking conditions. Student’s $t$ test (independent, two-tailed), $P < 0.001$ for all brain regions listed. Data presented as means ± SD, $n = 5$.

Figure 6  Ex vivo autoradiograms of $[^{18}F]9$ in the mouse brain under baseline (upper row) and self-blocking (with compound 9 at 3 $\mu$mol/kg, lower row) conditions: (A) Cortex; (B) Hippocampus; (C) Midbrain; (D) Cerebellum; (E) Lateral ventricle; (F) Striatum; (G) Thalamus; (H) Olfactory bulb.
the mouse pancreas, although high activity level was observed in this organ.

4. Conclusions

Through structure—activity relationship studies, indole-based compounds with the 5,6-dimethoxyisoindoline pharmacophore, a four-carbon linker, and a fluoroethoxy substituent in the indole moiety were discovered to confer favorable properties as selective $\sigma_2$ receptor ligands. Three radioligands with the $[^{18}F]$fluoroethoxy group at different positions of the indole ring were prepared and found to readily enter the brain, thus fulfilling the first requirement as neuroimaging agents. Further ex vivo and in vivo evaluations identified radiotracer $[^{18}F]9$, with the highest in vitro binding affinity and subtype selectivity for $\sigma_2$ receptor, as the most favorable agent for neuroimaging. In rodents it exhibited the highest levels of brain uptake, brain-to-blood ratio, and specific binding among the $\sigma_2$ receptor radioligands reported to date. Combined with its appropriate kinetics in the brain, the novel radioligand $[^{18}F]9$ from the current study is undoubtedly the most promising radioligand for neuroimaging of the $\sigma_2$ receptors, and thus warrants further evaluation in primates for its potential to image and quantitate cerebral $\sigma_2$ receptors in various brain diseases.

5. Experimental

5.1. General information

The reagents and solvents, thin-layer chromatography (TLC), $^1$H NMR and $^{13}$C NMR spectra, mass spectrometry (MS), high-resolution mass spectrometry (HRMS), HPLC, mice were obtained and handled in a manner similar to that reported in previous work. Details are provided in the Supporting Information. All the final compounds were characterized by $^1$H NMR, $^{13}$C NMR and HRMS. Chemical purities of $\geq 95\%$ were indicated by NMR spectra and analysis with HPLC (see Supporting Information). Normal male ICR mice (20 ± 2 g, 4–5 weeks) and male SD rats (200 ± 10 g, 6–7 weeks) were purchased from Vital River Experimental Animal Technical Co., Ltd. Experiments in animals were carried out in compliance with relevant regulations and institutional guidelines, and approved by the Institutional Animal Care and Use Committee of Beijing Normal University.

5.2. Radiochemistry

Synthesis of radiotracers, determination of logD value and in vitro stability studies followed the procedures reported previously. Detailed procedures are provided in the Supporting Information.

5.3. Biological evaluations

In vitro radioligand competition studies, in vivo biodistribution and blocking studies, effect of P-gp on brain uptake, ex vivo autoradiography, in vivo micro-PET/CT imaging...
in rats and in vivo metabolism studies followed the procedures reported previously. Detailed procedures are provided in the Supporting Information.

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Author contributions

All authors contributed to this manuscript and have approved the final article. Ying Zhang, Yiyun Huang and Hongmei Jia designed the study, participated in data analysis and interpretation and wrote the manuscript. Ying Zhang, Tao Wang and Xiaojun Zhang performed experiments. Winnie Deuther-Conrad and Peter Brust performed in vitro radioligand competition binding studies and participated in data analysis and interpretation. Hualong Fu, Mengchao Cui and Jinming Zhang designed and participated in experiments.

Conflicts of interest

The authors have no conflicts of interest to declare.

Appendix A. Supporting information

Supporting information to this article can be found online at https://doi.org/10.1016/j.apsb.2021.08.029.

References

1. Gabitova L, Gorin A, Astsaturov I. Molecular pathways: sterols and receptor signaling in cancer. Clin Cancer Res 2014;20:28–34.
2. Yang K, Zeng C, Wang C, Sun M, Yin D, Sun T. Sigma-2 receptor—a potential target for cancer/Alzheimer’s disease treatment via its regulation of cholesterol homeostasis. Molecules 2020;25:5439.
3. Chang TY, Yamauchi Y, Hasan MT, Chang C. Cellular cholesterol homeostasis and Alzheimer’s disease. J Lipid Res 2017;58:2239–54.
4. Matsushita Y, Nakagawa H, Koike K. Lipid metabolism in oncology: why it matters, how to research, and how to treat. Cancers 2021;13:474.
5. Mayengbam SS, Singh A, Pillai AD, Bhat MK. Influence of cholesterol on cancer progression and therapy. Transl Oncol 2021;14:101043.
6. Wellington CL. Cholesterol at the crossroads: Alzheimer’s disease and lipid metabolism. Clin Genet 2004;66:1–16.
7. Lukisz WJ, Pappolla M, Pelaez RP, Bazan NG. Alzheimer’s disease—a dysfunction in cholesterol and lipid metabolism. Cell Mol Neurobiol 2005;25:475–83.
8. Alon A, Schmidt HR, Wood MD, Sahn JJ, Martin SF, Kruse AC. Identification of the gene that codes for the sigma2 receptor. Proc Natl Acad Sci U S A 2017;114:7160–5.
9. Bartz F, Kern L, Erz D, Zhu M, Gilbert D, Meinhof T, et al. Identification of cholesterol-regulating genes by targeted RNAi screening. Cell Metab 2009;10:63–75.
10. Shen H, Li J, Xie X, Yang H, Zhang M, Wang B, et al. BRD2 regulation of sigma-2 receptor upon cholesterol deprivation. Life Sci Alliance 2020;4:e201900540.
11. Zeng C, Riad A, Mach RH. The biological function of sigma-2 receptor/TMEM97 and its utility in PET imaging studies in cancer. Cancers 2020;12:1877.
12. Mach RH, Smith CR, Al-Nabulsi I, Whirrett BR, Childers SR, Wheeler KT. Sigma2 receptors as potential biomarkers of proliferation in breast cancer. Cancer Res 1997;57:156–61.
13. Al-Nabulsi I, Mach RH, Wang LM, Wallen CA, Keng PC, Sten K, et al. Effect of ploidy, recruitment, environmental factors, and tamoxifen treatment on the expression of sigma-2 receptors in proliferating and quiescent tumour cells. Br J Cancer 1999;81:925–33.
14. Wheeler KT, Wang LM, Wallen CA, Childers SR, Cline JM, Keng PC, et al. Sigma2 receptors as a biomarker of proliferation in solid tumours. Br J Cancer 2000;82:1223–32.
15. Riad A, Zeng C, Weng CC, Winters H, Xu K, Makvandi M, et al. Sigma-2 receptor/TMEM97 and PGRMC1 increase the rate of internalization of LDL by LDL receptor through the formation of a ternary complex. Sci Rep 2018;8:16845.
16. Riad A, Lengyel-Zhand Z, Zeng C, Weng CC, Lee VM, Trojanowski JQ, et al. The sigma-2 receptor/TMEM97, PGRMC1, and LDL receptor complex are responsible for the cellular uptake of Abeta42 and its protein aggregates. Mol Neurobiol 2020;57:3803–13.
17. Izzo NJ, Xu J, Zeng C, Kirk MJ, Mozzoni K, Silicy C, et al. Alzheimer’s therapeutics targeting amyloid beta 1-42 oligomers II: sigma2/PGRMC1 receptors mediate Abeta 42 oligomer binding and synaptotoxicity. PLoS One 2014;9:e111899.
18. Izzo NJ, Staniszewski A, To L, Fa M, Teich AF, Saeed F, et al. Alzheimer’s therapeutics targeting amyloid beta 1-42 oligomers I: Abeta 42 oligomer binding to specific neuronal receptors is displaced by drug candidates that improve cognitive deficits. PLoS One 2014;9:e111898.
Brain-penetrant 18F-labeled radioligands for neuroimaging of the sigma-2 receptors

19. Grundman M, Morgan R, Lickliter JD, Schneider LS, DeKosky S, Izzo NJ, et al. A phase 1 clinical trial of the sigma-2 receptor complex allosteric antagonist CT1812, a novel therapeutic candidate for Alzheimer’s disease. *Alzheimers Dement* 2019;5:20–6.

20. Izzo NJ, Yuende CM, LaBarbera KM, Linegrover CS, Rehak C, Yurko R, et al. Preclinical and clinical biomarker studies of CT1812: a novel approach to Alzheimer’s disease modification. *Alzheimers Dement* 2021;17:1365–82.

21. Huang YS, Lu HL, Zhang LJ, Wu Z. Sigma-2 receptor ligands and their perspectives in cancer diagnosis and therapy. *Med Res Rev* 2014;34:532–66.

22. Mach RH, Zeng C, Hawkins WG. The σ2 receptor: a novel protein for the imaging and treatment of cancer. *J Med Chem* 2013;56:7137–60.

23. Zeng C, McDonald ES, Mach RH. Molecular probes for imaging the σ2 receptor: *in vitro* and *in vivo* imaging studies. *Handb Exp Pharmacol* 2017;244:309–30.

24. Dehdashti F, Laforest R, Gao F, Shoghi KI, Aft RL, Nussenbaum B, et al. Assessment of cellular proliferation in tumors by PET using 18F-ISO-1 uptake as a marker of proliferation status. *J Nucl Med* 2020;61:665–70.

25. Abramson Cancer Center of the University of Pennsylvania. Imaging of *in vivo* sigma-2 receptor expression with 18F-ISO-1 positron emission tomography in metastatic breast cancer. *Clinical Trials.gov Identifier* NCT03057743.

26. Tu Z, Xu J, Jones LA, Li S, Dunstorff C, Vangveravong S, et al. Flurorine-18-labeled benzamide analogues for imaging the σ2 receptor status of solid tumors with positron emission tomography. *J Med Chem* 2007;50:3194–204.

27. Mesangeau C, Amata E, Alsharif W, Seminero MJ, Robson MJ, Matsumoto RR, et al. Synthesis and pharmacological evaluation of indole-based sigma receptor ligands. *Eur J Med Chem* 2011;46:5154–61.

28. Wang L, Ye J, He Y, Deather-Conrad W, Zhang J, Zhang X, et al. 18F-Labeled indole-based analogs as highly selective radioligands for imaging sigma-2 receptors in the brain. *Bioorg Med Chem* 2017;25:3792–802.

29. Fan C, Jia H, Deather-Conrad W, Brust P, Steinbach J, Liu B. Novel 99mTc labeled σ receptor ligand as a potential tumor imaging agent. *Sci China Ser B Chem* 2006;49:169–76.

30. Bautista-Aguilera OM, Budni J, Mina F, Medeiros EB, Deather-Conrad W, Entremont JM, et al. Contilisant, a tetra target small molecule for Alzheimer’s disease therapy combining cholinesterase, monoamine oxidase inhibition, and H3R antagonism with S1R agonist profile. *J Med Chem* 2018;61:6937–43.

31. Li Y, Wang X, Zhang J, Deather-Conrad W, Xie F, Zhang X, et al. Synthesis and evaluation of novel 18F-labeled spirocyclic piperidine derivatives as σ1 receptor ligands for positron emission tomography imaging. *J Med Chem* 2013;56:3478–91.

32. Patel S, Gibson R. *In vivo* site-directed radiotracers: a mini-review. *Nucl Med Biol* 2008;35:805–15.

33. Intagliata S, Sharma A, King TI, Mesangeau C, Seminero M, Chin FT, et al. Discovery of a highly selective sigma-2 receptor ligand, 1-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-3-methyl-1H-benzof[di]imidazole-2(3H)-one (CM398), with drug-like properties and antinociceptive effects in *vivo*. *AAPS J* 2020;22:94.

34. Kreisl WC, Liow JS, Kimura N, Seneca N, Zoghibi SS, Morse CL, et al. P-glycoprotein function at the blood–brain barrier in humans can be quantified with the substrate radiotracer 11C-N-desmethylloperamide. *J Nucl Med* 2010;51:559–66.

35. Abate C, Selivanova SV, Muller A, Kramer SD, Schibli R, Marottoli R, et al. Development of 3,4-dihydroisoquinolin-1(2H)-one derivatives for the Positron Emission Tomography (PET) imaging of σ2 receptors. *Eur J Med Chem* 2013;69:920–30.

36. Tian J, He Y, Deather-Conrad W, Fu H, Xie F, Zhang Y, et al. Synthesis and evaluation of new 1-oxa-8-azaspiro[4.5]decane derivatives as candidate radioligands for sigma-1 receptors. *Bioorg Med Chem* 2020;28:115560.

37. Sahn JJ, Mejia GL, Ray PR, Martin SF, Price TJ. Sigma 2 receptor (Tmem97) agonists produce long lasting antineuropathic pain effects in mice. *ACS Chem Neurosci* 2017;8:1801–11.

38. Lever JR, Miller DK, Green CL, Ferguson-Cantrell EA,Watkinson LD, Carmack TL, et al. A selective sigma-2 receptor ligand antagonizes cocaine-induced hyperlocomotion in mice. *Synapse* 2014;68:73–84.

39. Soby KK, Mikkelsen JD, Meier E, Thomson C. Lu 28-179 labels a σ1 receptor site in rat and human brain. *Neuropharmacology* 2002;43:95–100.

40. He Y, Xie F, Ye J, Deather-Conrad W, Cui B, Wang L, et al. 1-(4-[18F]Fluorobenzyl)-(tetrahydrofuran-2-yl)methyl]piperazine: a novel suitable radioligand with low lipophilicity for imaging σ1 receptors in the brain. *J Med Chem* 2017;60:4161–72.

41. Chen YY, Wang X, Zhang JM, Deather-Conrad W, Zhang XJ, Huang Y, et al. Synthesis and evaluation of a 18F-labeled spirocyclic piperidine derivative as promising σ1 receptor imaging agent. *Bioorg Med Chem* 2014;22:5270–8.

42. Leitner ML, Hohmann AG, Patrick SL, Walker JM. Regional variation in the ratio of σ1 to σ2 binding in rat brain. *Eur J Pharmacol* 1994;259:65–9.