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Radiosynthesis and evaluation of a fluorine-18 labeled radioligand targeting vesicular acetylcholine transporter

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ABSTRACT

Vesicular acetylcholine transporter (VAChT) is a reliable biomarker for assessing the loss of cholinergic neurons in the brain that is associated with cognitive impairment of patients. 5-Hydrotetralin compound **(±)-5-OH-VAT** is potent (K_i = 4.64 ± 0.32 nM) and selective for VAChT (>1800-fold and 398-fold for σ_1 and σ_2 receptor) with favorable hydrophilicity with LogD value of 1.78, although **(-)-5-OH-VAT** originally serves as the radiolabeling precursor of (-)-[^{18}F]VAT, a promising VAChT radiotracer with logD value of 2.56. To evaluate **(-)-5-OH-[^{18}F]VAT** as a radiotracer for VAChT, we performed *in vitro* assay to determine the potency of the minus enantiomer **(-)-5-OH-VAT** and plus enantiomer **(+)-5-OH-VAT**, indicating that **(-)-5-OH-VAT** is a more potent VAChT enantiomer. Radiosynthesis of **(-)-5-OH-[^{18}F]VAT** was explored using three strategies. **(-)-5-OH-[^{18}F]VAT** was achieved with a good yield (24 ± 6%) and high molar activity (~ 37 GBq/μmol, at the end of synthesis) using a microwave assist two-step one-pot procedure that started with di-MOM protected nitro-containing precursor (-)-**6**. MicroPET studies in the brain of nonhuman primate (NHP) suggest that **(-)-5-OH-[^{18}F]VAT** readily penetrated the blood brain barrier and specifically accumulated in the VAChT-enriched striatum with improved washout kinetics from striatum compared to [^{18}F]VAT. Nevertheless, the lower target to non-target ratio may limit its use for *in vivo* measure the VAChT level in the brain.

The vesicular acetylcholine transporter (VAChT) is a neurotransmitter transporter consisting of 550 amino acid polypeptides.[1, 2] VAChT is located on the presynaptic vesicular membrane of cholinergic neurons.[3] One main function of the VAChT is loading neurotransmitter acetylcholine, which plays an important role in learning, memory, and motivation, into presynaptic vesicles. When the presynaptic terminal is activated, acetylcholine is released into the synaptic cleft and signals through two classes of receptors, nicotinic acetylcholine receptors and muscarinic acetylcholine receptors. It's widely accepted that depletion in VAChT expression levels in the central nervous system (CNS) has been correlated with the occurrence and severity of dementia progression.[4-6] VAChT is a reliable cholinergic marker for quantitative assessment of the cognitive impairment for patients with dementia such as Alzheimer's disease (AD), Parkinson's disease (PD).

Positron emission tomography (PET) or single-photon emission computed tomography (SPECT) is a noninvasive and sensitive imaging modality for mapping of cholinergic function in the brain. Significant advances are achieved in the understanding of neurobiology and pathophysiology of dementia with PET/SPECT imaging modalities.[7-9] Currently, most developed radiotracers are based on *trans*-2-(4-phenyl-piperidino)cyclohexanol (vesamicol) scaffold.[4,6] Investigators have put tremendous efforts in the development of suitable tracers for mapping VAChT densities in human brain.[10-12] However, limited tracers are used for clinical investigation. This is due to the fact that many reported VAChT radioligands show poor selectivity over sigma receptors, which are extensively expressed throughout the brain. Furthermore, other factors such as slow brain equilibrium kinetics, fast metabolism, and high plasma protein binding limit their clinical utility. Up to date, only three radiotracers are approved for imaging VAChT levels in the human brain (**Figure 1**). (-)-5-[^{123}I]iodo-benzovesamicol ([^{123}I]IBVM) is the first SPECT radiotracer used for mapping VAChT densities in the human subjects and the uptake of [^{123}I]IBVM in the target regions peaked at least 6 h post-injection (p.i.).[10, 13-15] Fluorine-18 labeled (-)-5-[^{18}F]fluoroethoxybenzovesamicol ([^{18}F]FEOBV) is the first PET radioligand to assess VAChT levels in human with high specificity and sensitivity.[11, 16, 17] *Ex vivo* brain autoradiography of [^{18}F]FEOBV in human AD cases and age-matched controls showed an apparent difference of cholinergic densities.[17] *In vivo* imaging analysis indicated a good correlation between the reference tissue modeling and the full kinetic

compartment modeling with arterial sampling as well as plasma metabolite analysis. Similar to the SPECT radiotracer [^{123}I]IBVM, the fluorine-18 labeled [^{18}F]FEOBV PET also requires over 6 h to equilibrate in the striatum. Nonetheless, [^{18}F]FEOBV had low binding in the lateral cerebellar cortex while with high uptake in the mesopontine junction and medulla, which further support a strong index of VAChT binding. Furthermore, PET is superior to SPECT in terms of detection efficiency, spatial resolution, and quantification.[18, 19] Most recently, our group reported a new radiotracer (-)-(1-(8-(2-[^{18}F]fluoroethoxy)-3-hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-piperidin-4-yl)(4-fluorophenyl)-methanone ([^{18}F]VAT) [12, 20-29] possessing promising radiopharmaceutical profiles in rodents and nonhuman primates (NHPs), favorable *in vivo* radiation dosimetry.[30] We successfully produced [^{18}F]VAT in a current Good Manufacturing Practice (cGMP) facility with reliable yields and clinical release criteria. Subsequently, we obtained exploratory Investigational New Drug (exploratory IND) approval of [^{18}F]VAT from the Food and Drug Administration (FDA). The investigation of [^{18}F]VAT for CNS diseases with cholinergic depletion in human is actively ongoing. [^{18}F]VAT is a significant complement to currently developed presynaptic cholinergic imaging agents.

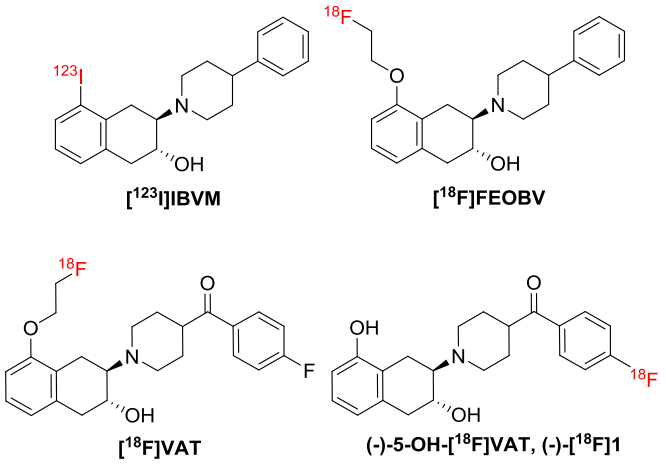


Figure 1. Structures of [^{123}I]IBVM, [^{18}F]FEOBV, and [^{18}F]VAT and **(-)-5-OH-[^{18}F]VAT**.

Racemic compound **(±)-5-OH-VAT**, (±)-**1** has good binding affinity (K_i = 4.64 ± 0.32 nM) for VAChT and very low binding affinity for σ_1 receptors (K_i = 8,640 ± 974 nM), and σ_2 receptors (K_i = 1,851 ± 408 nM). Upon removing the 5-(2-fluoroethyl) group from VAT, the free hydroxyl group retained in the structure of **5-OH-VAT** improves the hydrophilicity (LogD = 1.78) compared to VAT (LogD = 2.56). More importantly, a fluorine group in the para-position of the benzoyl moiety allows incorporation of a fluorine-18 by aromatic nucleophilic substitution reaction. To identify a better VAChT imaging tracer for assessing the loss of cholinergic neurons in the brain of patient with neurodegenerative disease, we decided to investigate the 5-hydroxytetralin compound (±)-**5-OH-VAT**. After the optically pure **(-)-5-OH-VAT** and **(+)-5-OH-VAT** were obtained, *in vitro* assay was performed to assess the potencies of each enantiomer binding to VAChT, σ_1 and σ_1 receptors, indicating **(-)-5-OH-VAT** is the more potent enantiomer. In order to perform the *in vivo* evaluation of **(-)-5-OH-[^{18}F]VAT**, three strategies were explored to produce **(-)-5-OH-[^{18}F]VAT**, suggesting that using a microwave assisted two-step one-pot procedure is able to provide sufficient dose for further *in vivo* PET evaluation in the brain of nonhuman primate.

The synthesis of the target VAChT compound **1** (**5-OH-VAT**) was conducted according to our published procedure.[23] The (+)-**1** and (-)-**1** enantiomers were obtained by chiral semi-preparative separation and both enantiomers displayed enantiomeric excess value >95%. The competitive binding assay results for the optically pure (+)-**1**, (-)-**1**, and racemic mixtures towards VAChT are depicted in **Table 1**. Tritiated vesamicol was used as the competitive radioligand. Since binding selectivity for VAChT over σ_1 and σ_2 is one of the major challenges in the development of VAChT PET radiotracers, we also conducted *in vitro* binding assay towards σ_1 and σ_2 receptors using [^{3}H]pentazocine and [^{3}H]ditolylguanidine ([^{3}H]DTG) as the radioligand, respectively. *In vitro* data showed that the minus enantiomer (-)-**1** ($K_{i\text{-VAChT}}$ = 4.62 nM) is slightly more potent than the racemic mixtures ($K_{i\text{-VAChT}}$ = 4.64 nM) and its (+)-counterpart for VAChT ($K_{i\text{-VAChT}}$ = 5.27 nM). All compounds display binding affinities around 5 nM for VAChT, which was about 8-fold less potent than our earlier developed (-)-[^{18}F]VAT ($K_{i\text{-VAChT}}$ = 0.59 nM), indicating the fluoroethoxy group in the 5-postion of the tetralin ring impacts the VAChT binding affinity. Furthermore, the racemic mixtures and enantiomers show low binding affinities for both σ_1 and σ_2 receptors with selectivity over 300-fold, which is consistent with reported analogues.[20, 22, 23] Removal of the fluoroethyl group from VAT, the lipophilicity of compound **1** (LogD = 1.78) was decreased compared to VAT (LogD = 2.56), suggesting that compound **1** is favorable in penetrating the blood brain barrier of animal.[31] In combination of the binding affinity, fast washout kinetics of **(-)-5-OH-[^{18}F]VAT** compared to [^{18}F]VAT are expected.

With the binding data in hand, we focus on the synthesis of the radiolabeling precursor and radiosynthesis of [^{18}F]**1**. Reaction of epoxy **2** with piperidine hydrochloride **3**, which were prepared according to our previously reported procedures,[23] afforded two regioisomers (±)-**4a** and (±)-**4b** in 54% and 30% yield, respectively (**Scheme 1**). It's reported that compound (±)-**1** with the hydroxyl group in the 5-position of the tetralin ring is more potent than corresponding regioisomer with the hydroxyl group in the 8-position of the tetralin ring for VAChT.[23] Next, we put our effort on the radiosynthesis of [^{18}F]**1**, a VAT analogue with a 5-hydroxyl group in the tetralin moiety. In search of a promising VAChT radioligand, previously we reported a one-step fluorine-18 radiolabeling of VAChT compound with very similar structure as (±)-**4a**.[20] Our first strategy was to use a one-step aromatic nucleophilic -NO_2/[^{18}F]F^- substitution method that started with the nitro-containing precursor (±)-**4a.** Reaction of (±)-**4a** with K[^{18}F]/F^- in DMSO at 120 °C for 20 min, only trace product was observed (0.3% decay corrected yield). Increasing the reaction time or the base amount had no improvement on the product yield (< 1% decay corrected yield). We envisioned that one more hydroxyl group was introduced to the tetralin ring compared to our earlier developed tracer, the acidic hydroxyl group on the tetralin ring probably interfered with the reaction. Therefore, we used two acetyl groups to protect the two hydroxyl groups in (±)-**4a** that generated nitro-containing precursor compound (±)-**5** in quantitative yield (**Scheme 1**). Our second strategy was to use two acetyl group protected nitro-containing precursor (±)-**5** reacting with K[^{18}F]/F^- in DMSO at 120 °C, followed by hydrolysis of both acetyl groups to afford (±)-[^{18}F]**1** with slightly improved yield (2% decay corrected to EOS). For this strategy, it was observed that most precursor decomposed under the reaction conditions. No improvement on the product yield was observed when increasing the reaction temperature (**Scheme 2A**). Stepwise reaction further showed the first step gave low [^{18}F]/fluoride incorporation. The low conversion is probably due to the competitive reaction between nitro group substitution with fluoride and hydrolysis of the acetyl groups under strong basic and high temperature

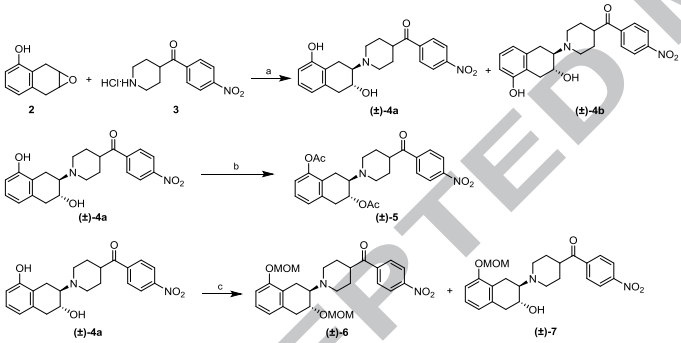
.

Table 1. Binding affinities of VAChT compounds towards VAChT, σ_1, and σ_1 receptors.

Ligands	$K_{i\text{-}VAChT}$ (nM)	$K_{i\text{-}\sigma1}$ (nM)	$K_{i\text{-}\sigma2}$ (nM)	VAChT/σ_1	VAChT/σ_2	$LogD_{pH\ 7.4}$ [a] Calculated	$LogD_{pH\ 7.4}$ [b] Experimental
(±)-1[-]	4.64 ± 0.32	8,640 ± 974	1,851 ± 408	1,862	398		
(+)-1	5.27 ± 0.21	3076 ± 680	> 10,000	757	1898	2.63	1.78 ± 0.13
(-)-1	4.62 ± 0.22	2012 ± 450	> 10,000	435	2165		
(-)-VAT[c]	0.59 ± 0.06	>10,000	>10,000	>10,000	>10,000	3.45	2.56 ± 0.15

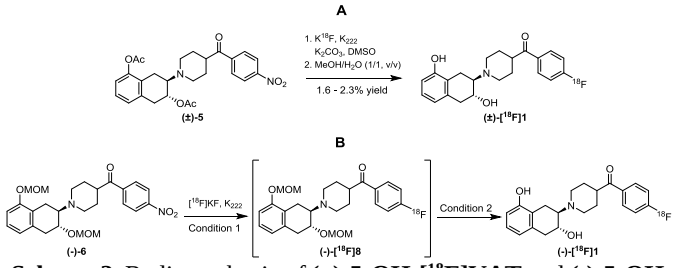
[a] Calculated value at pH = 7.4 by ACD/LogD 7.0 (Advanced Chemistry Development, Inc., Canada); [b] measured according to reference 22; [c] reference 23.

conditions. This may result from the hydrolysis prior to aromatic nucleophilic substitution reaction between $-NO_2/[^{18}F]$/fluoride. The acetyl group might not stable when heated under the base conditions. We changed the protection from acetyl group to (methoxymethyl) MOM group via (±)-**4a** reacting with methoxymethyl chloride (MOMCl) that afforded di-MOM protected (±)-**6** and mono-MOM protected (±)-**7** in 33% and 63% yield, respectively. Since the binding of compounds toward VAChT is stereoselective and generally for the vesamicol analogue, the minus enantiomer is more potent than the plus counterpart. Therefore, we tried to resolve the enantiomers using a Chiralcel OD column. For mono-MOM protected compound (±)-7, it was very challenging to resolve using normal phase chiral high performance liquid chromatography (HPLC) column. Anyway, di-MOM protected racemic mixture (±)-**6** was smoothly resolved to generate the minus enantiomer (-)-**6** and plus enantiomer (+)-**6** (**Scheme 1**).



Scheme 1. Synthesis of precursors (±)-**4a**, (±)-**5**, and (±)-**6**. *Reagents and conditions:* (a) Et_3N, EtOH, reflux, 54% for (±)-**4a**, 30% for (±)-**4b**; (b) Ac_2O, pyridine, CH_2Cl_2, quantitative yield; (c) MOMCl, $^iPr_2NEt$, CH_2Cl_2, 33% for (±)-**6**, 63% for (±)-**7**.

With the minus enantiomer (-)-**6** in hand, our third strategy was to use the di-MOM protected nitro-containing precursor (-)-**6** reacting with dried $K[^{18}F]/F^-$ in DMSO. To evaluate fluorine-18 incorporation yield in the first step, the standard reference intermediate (±)-**8** was prepared by reaction of **(±)-5-OH-VAT** with MOMCl in moderate yield. To achieve a reasonable yield of **(-)-5-OH-[^{18}F]VAT**, different reaction conditions using precursor (-)-**6** were investigated. The reaction conditions and results were outlined in **Table 2**. Firstly, we tried to use conventional oil-bath heat method to make (-)-[^{18}F]**8**. Reaction of (-)-**6** with dried K $[^{18}F]/F^-$ in DMSO at 140 °C for 15 min gave 32% conversion of $[^{18}F]/F^-$, further acidolysis of both protecting groups afforded the product **(-)-5-OH-[^{18}F]VAT** in 7% decay corrected to EOS (**Table 2**, entry 1). The yield was improved, but inadequate to generate sufficient dose for PET study on the NHP brain. Then, we switched the oil-bath heating to microwave irradiation. When the reaction mixture was heated 40 seconds at 60 watts power, an 18% conversion was observed; increasing the reaction time to 3 × 40 seconds led to higher fluorine-18 incorporation (27%); using DMF as the solvent, elevating the power to 65 watts and extending the reaction time to 3 × 90 seconds resulted in only 15% incorporation; a 2% yield was achieved for the final product **(-)-5-OH-[^{18}F]VAT** after acidolysis of both MOM protection groups using 6 M aqueous hydrochloric acid solution under microwave condition (**Table 2**, entries 2 - 4). Nevertheless, assisted with microwave, higher fluorine-18 conversion (40%) was achieved when the reaction was conducted at lower power (50 watts) and extended reaction time (120 seconds × 3) with DMF as the solvent. The yield of **(-)-5-OH-[^{18}F]VAT** was significantly improved after acidolysis of both MOM protecting groups (24 ± 6%). Generally, starting with ~5.6 GBq dried fluoride, 0.5 – 1 GBq of **(-)-5-OH-[^{18}F]VAT** was easily obtained with excellent radiochemical purity and high molar activity (~ 37 GBq/μmol) (**Table 2**, entry 5). Furthermore, it was observed that the product radioactivity chromatogram of the microwave assisted two-step procedure had fewer impurities than the conventional oil-bath heating condition, suggesting that microwave assistant radiolabeling was highly efficient for both steps under short reaction time.



Scheme 2. Radiosynthesis of **(±)-5-OH-[^{18}F]VAT** and **(-)-5-OH-[^{18}F]VAT**.

Table 2. Condition screening for the radiosynthesis of (-)-[^{18}F]**1**. [a]

Entry	Condition 1	Conversion for (-)-[^{18}F]**8** [b]	Condition 2	Yield for two steps	Product (@EOS)
1	DMSO, 140 °C, 15 min	32%	CF_3COOH, 140 °C, 10 min	7%	0.2 GBq
2	DMSO, MW 60 W, 40 s	18%	N.P.[b]	N.P.	N.P.
3	DMSO, MW 60 W, 40 s × 3	27%	N.P.	N.P.	N.P.
4	DMF, MW 65 W, 90 s × 3	15%	6 M HCl, MW 50 W, 120 s × 3	2%	0.015 GBq

5	DMF, MW 50 W, 120 s × 3	40%	6 M HCl, MW 50 W, 120 s × 2	24 ± 6%	0.5 – 1 GBq [c]

[a] N.P., reaction not performed; MW, microwave; EOS, end of synthesis; [b] Monitored by radioTLC; [c] the reaction was conducted for three times.

MicroPET study of **(-)-5-OH-[^{18}F]VAT** in the brain of an adult male M. fascicularis indicated this radiotracer penetrated the animal blood brain barrier well and the highest accumulation was observed in the VAChT-enriched striatal region. The uptake of **(-)-5-OH-[^{18}F]VAT** in the NHP brain peaked at 7 min followed by a fast washout from all brain regions (**Figure 2**). Relatively high uptake was observed in the thalamus and frontal cortex, whereas lower uptake was observed in the cerebellum. Compared to [^{18}F]VAT, **(-)-5-OH-[^{18}F]VAT** displayed slightly higher standardized uptake value (SUV) and earlier peak uptake (6.2 at 7 min vs 5.1 at 15 min);[23] as expected, **(-)-5-OH-[^{18}F]VAT** showed faster brain kinetics in the target striatal region than [^{18}F]VAT. The faster kinetics might attribute to the improved hydrophilicity of **(-)-5-OH-[^{18}F]VAT** and relative low VAChT binding potency compared to [^{18}F]VAT. The SUV change may be due to the molar activity difference of the two tracers, or may be attributed to the difference lipophilicity of these two radiotracers. The experimental measure of the log D values of both radiotracers are lower than the calculated Log D values (Table 1). The uptake ratio of the target (striatum) to non-target (cerebellum) increased gradually during the scan and reach about 2 at 35 min p.i., which remained stable until 120 min and was slightly lower than [^{18}F]VAT (the ratio was 3.0). The lower target to non-target ratio may prevent it to be a better VAChT radiotracer than [^{18}F]VAT, even though **(-)-5-OH-[^{18}F]VAT** displayed faster brain kinetics.

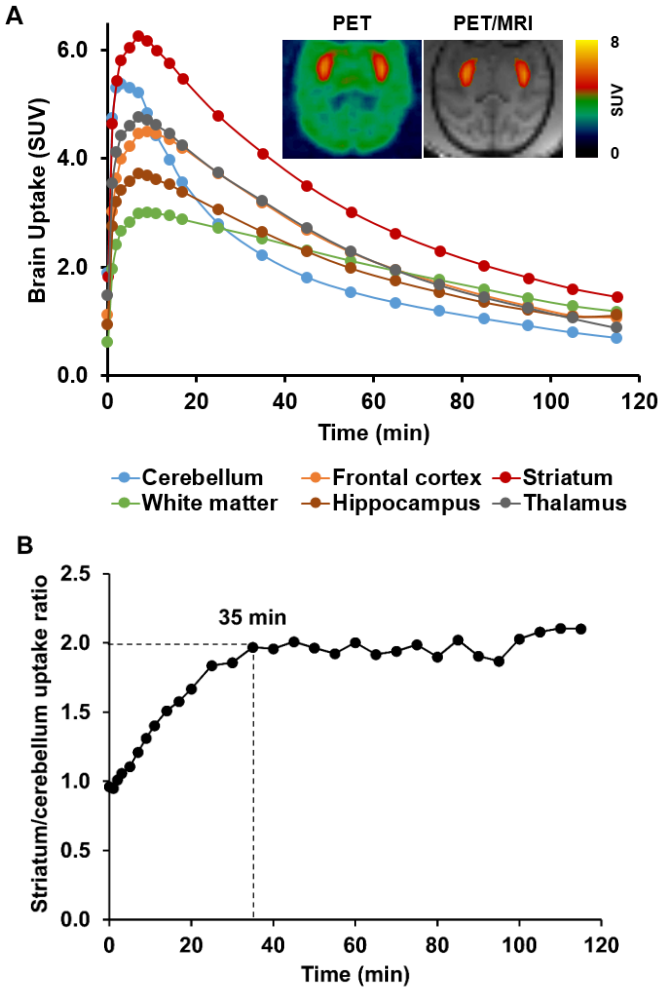


Figure 2. PET study of **(-)-5-OH-[^{18}F]VAT** in a male M. fascicularis brain. (A) Representative PET images and time-activity curve demonstrate the specific binding of **(-)-5-OH-[^{18}F]VAT** to VAChT-enriched striatum. (B) The striatum/cerebellum uptake ratio curve shows that the ratio reaches about 2 at 35 min p.i., and remained stable until 120 min.

Experimental details for the radiolabeling precursors including chiral resolution, standard reference, radiolabeling procedures, and binding assay methods are provided in the Notes.[32]

In the current study, we evaluated a new VAChT radioligand (-)-[^{18}F]**1** ((-)-5-OH-[^{18}F]**VAT**), an analogue of [^{18}F]VAT; (-)-**1** show high binding affinity towards VAChT and good selectivity over σ_1 and σ_2 receptors. After extensive exploration, (-)-[^{18}F]**1** was successfully synthesized by a two-step one-pot microwave-assisted reaction strategy in high yield and good molar activity. Preliminary imaging results in an NHP indicated (-)-[^{18}F]**1** had fast clearance kinetics from the target striatal region of the brain compared to [^{18}F]VAT, but the lower target to non-target ratio may prevent it to be a suitable PET radiotracer for quantitative measurement of the VAChT in human brain.

Acknowledgements

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32. Experimental section. (A) *General*: All solvents and reagents were purchased commercially and were used as received unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) using silica gel 60 F254 glass plates (EMD Chemicals Inc.). Flash column chromatography was performed over silica gel (32-63 μm), HPLC grade solvents were used for chromatography. ^{1}H NMR and ^{13}C NMR spectra were carried out on a Varian Mercury-VX 400 MHz spectrometer. The ^{13}C NMR spectra were recorded at 125 MHz. The chemical shifts are reported as δ values (ppm) relative to tetramethylsilane (TMS) as an internal reference. The Spectra System was used for both analytical and semi-preparative HPLC. A Chiralcel OD normal phase HPLC column was used to separate the enantiomeric isomers from the racemic compounds. The optical rotation for each enantiopure compound was determined on an automatic polarimeter (Autopol 111, Rudolph Research, Flanders, NJ). *(1-(3,8-Dihydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)piperidin-4-yl)(4-nitrophenyl)methanone ((±)-**4a**) and (1-(3,5-dihydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)piperidin-4-yl)(4-nitrophenyl)-methanone ((±)-**4b**):* To a solution of compounds **2** (0.58 g, 3.6 mmol) in ethanol (15 mL) was added Et_3N (1.8 g, 18 mmol) and (4-nitrophenyl)(piperidin-4-yl)methanone hydrochloride **3** [22, 20, 33] (0.81 g, 3.0 mmol) successively. The solution was heated to reflux for 60 hours. TLC showed that the starting material was consumed completely. The solution was concentrated and the residue was subjected to silica gel chromatography using hexane/EtOAc/Et_3N (1/1/5%, v/v/v) as the eluent to afford two separable regioisomers (±)-**4a** (0.65 g, 54% yield) and (±)-**4b** (0.36 g, 30% yield). (±)-**4a:** yellow solid, mp 217 – 218 °C. ^{1}H NMR (400 MHz, CD_3SOCD_3) δ 9.37 (s, 1H), 8.35 (d, J = 8.8 Hz, 2H), 8.21 (d, J = 8.8 Hz, 2H), 6.89 (t, J = 7.7 Hz, 1H), 6.59 (d, J = 7.9 Hz, 1H), 6.51 (d, J = 7.5 Hz, 1H), 4.34 (s, 1H), 3.84 – 3.74 (m, 1H), 3.49 (t, J = 11.2 Hz, 1H), 3.05 – 2.95 (m, 1H), 2.92 – 2.80 (m, 3H), 2.79 – 2.57 (m, 4H), 2.49 – 2.38 (m, 1H), 1.83 (t, J = 12.6 Hz, 2H), 1.72 – 1.55 (m, 2H). ^{13}C NMR (101 MHz, CD_3SOCD_3) δ 202.3, 155.4, 150.2, 141.0, 136.0, 130.0, 126.6, 124.4, 122.5, 119.5, 112.1, 66.2, 66.1, 50.0, 46.5, 44.3, 38.8, 29.3, 29.2, 22.0. HRMS (ESI) Calcd for $C_{22}H_{24}N_2O_5$ ([M+H]$^+$) 397.1758, found: 397.1752. (±)-**4b:** yellow solid, mp 223 – 225 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.34 (d, J = 8.5 Hz, 2H), 8.20 (d, J = 8.6 Hz, 2H), 6.89 (t, J = 7.7 Hz, 1H), 6.56 (dd, J = 19.5, 7.7 Hz, 2H), 4.36 (s, 1H), 3.87 – 3.77 (m, 1H), 3.46 (t, J = 11.3 Hz, 1H), 3.12 – 3.01 (m, 1H), 2.95 – 2.58 (m, 7H), 2.37 – 2.24 (m, 1H), 1.87 – 1.76 (m, 2H), 1.68 – 1.51 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.9, 154.8, 149.8, 140.5, 136.7, 129.6, 126.1, 124.0, 121.2, 119.2, 111.7, 66.1, 65.2, 49.6, 46.4, 43.9, 32.5, 28.0, 28.7, 27.6. HRMS (ESI) Calcd for $C_{22}H_{24}N_2O_5$ ([M+H]$^+$) 397.1758, found: 397.1755.
*7-(4-(4-Nitrobenzoyl)piperidin-1-yl)-5,6,7,8-tetrahydronaphthalene-1,6-diyl diacetate ((±)-**5**):* To a mixture of **(±)-4a** (186 mg, 0.47 mmol) in dichloromethane (6 mL) was added acetic anhydride (0.30 mL) and pyridine (0.30 mL). The resulting mixture was stirred under room temperature overnight. Solvents were evaporated, the product was purified on a flash column chromatography (silica gel, hexane/ethyl acetate: 3/1 to 1/1) to provide (±)-**5** as brown liquid (233.7 mg, quantitative yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, J = 8.6 Hz, 2H), 8.04 (d, J = 8.9 Hz, 2H), 7.13 (t, J = 7.8 Hz, 1H), 6.95 (d, J = 7.6 Hz, 1H), 6.86 (d, J = 8.0 Hz, 1H), 5.31 – 5.20 (m, 1H), 3.26 – 3.13 (m, 2H), 3.05 – 2.92 (m, 3H), 2.91 – 2.79 (m, 2H), 2.68 – 2.57 (m, 1H), 2.52 (t, J = 11.2 Hz, 2H), 2.33 (s, 3H), 2.10 (s, 3H), 1.90 – 1.73 (m, 3H), 1.73 – 1.58 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.1, 170.4, 169.2, 150.1, 148.6, 140.7, 135.5, 129.2, 127.4, 126.9, 126.4, 123.89, 119.8, 69.3, 62.7, 49.3, 48.6, 44.6, 35.0, 29.2, 29.0, 23.5, 21.5, 20.9. HRMS (ESI) Calcd for $C_{26}H_{29}N_2O_7$ ([M+H]$^+$) 481.1969, found: 481.1788.
*(1-(3,8-Bis(methoxymethoxy)-1,2,3,4-tetrahydronaphthalen-2-yl)piperidin-4-yl)(4-nitrophenyl)-methanone ((±)-**6**): (1-(3-hydroxy-8-(methoxymethoxy)-1,2,3,4-tetrahydronaphthalen-2-yl)piperidin-4-yl)(4-nitrophenyl)methanone ((±)-**7**):* To a solution of compound (±)-**4a** (0.20 g, 1.1 mmol) in CH_2Cl_2 (10 mL) and THF (5 mL) cosolvents was added $^{i}Pr_2NEt$ (129 mg, 1.0 mmol). The solution was cooled down to 0 °C and chloromethyl methyl ether (61 mg, 0.75 mmol) was added dropwise. The reaction was warmed to room temperature upon addition and continued to stir for overnight. The reaction was quenched with water, extracted with CH_2Cl_2. The combined organic phase was washed with saturated sodium bicarbonate, saturated sodium chloride solution respectively, and dried with anhydrous sodium sulfate. The concentrated residue was subjected to silica gel chromatography using hexane/EtOAc/Et_3N (3/1/5%) as the eluent to afford the mono-MOM protected product (±)-**6** (0.16 g, 33% yield) and di-MOM protected compound (±)-**7** (0.28 g, 63% yield), 45 mg of the starting material was recovered. (Note: Two batches of reaction mixtures were combined together for purification). (±)-**6:** yellow solid, mp 122 – 124 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 8.0 Hz, 2H), 7.98 (d, J = 8.0 Hz, 2H), 6.98 (t, J = 7.8 Hz, 1H), 6.80 (d, J = 8.0 Hz, 1H), 6.66 (d, J = 7.8 Hz, 1H), 5.12 (s, 2H), 4.74 (dd, J = 21.6 Hz, 6.8 Hz, 2H), 3.94 – 3.88 (m, 1H), 3.40 (s, 3H), 3.36 (s, 3H), 3.23 – 3.08 (m, 2H), 3.02 – 2.92 (m, 4H), 2.79 – 2.71 (m, 1H), 2.63 – 2.55 (m, 3H), 1.82 – 1.78 (m, 3H), 1.77 – 1.65 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.2, 154.6, 150.1, 140.7, 135.9, 129.2, 126.7, 124.6, 123.9, 121.9, 111.0, 95.7, 94.3, 73.5, 64.2, 56.0, 55.8, 49.7, 47.7, 44.8, 36.2, 29.2, 29.0, 22.6. HRMS (ESI) Calcd for $C_{26}H_{33}N_2O_7$ ([M+H]$^+$) 485.2282, found: 485.2289. Enantiomeric resolution of compound (±)-**6** was accomplished

by HPLC using a 250 mm × 9.2 mm Chiralcel OD column with detection wavelength set at 254 nm, mobile phase consisting of hexane/^{i}PrOH /Et_3N (86/13/1, v/v/v), flow rate at 4 mL/min. The optical purity was determined by chiral analytical HPLC with the same mobile phase (Chiralcel OD column, 250 mm × 4.6 mm, 1 mL/min, UV 254 nm). The optical rotation of (-)-**6**: $[\alpha]_D^{20}$ = -13.9° (2.8 mg/mL in MeOH)

(±)-**7:** yellow solid, mp 141 – 143 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 8.8 Hz, 2H), 8.00 (d, J = 9.2 Hz, 2H), 7.00 (t, J = 8.0 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 6.68 (d, J = 8.0 Hz, 1H), 5.13 (s, 2H), 3.79 – 3.71 (m, 1H), 3.41 (s, 3H), 3.32 – 3.21 (m, 1H), 3.20 – 3.16 (m, 1H), 3.03 – 2.98 (m, 1H), 2.94 – 2.88 (m, 1H), 2.84 – 2.78 (m, 2H), 2.74 – 2.67 (m, 2H), 2.52 – 2.43 (m, 2H), 1.92 – 1.81 (m, 3H), 1.79 – 1.70 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.9, 155.0, 150.2, 140.6, 135.4, 129.2, 126.9, 124.1, 124.0, 122.4, 110.8, 94.3, 66.5, 65.2, 56.1, 51.6, 44.5, 44.4, 37.9, 29.2, 28.9, 20.3. HRMS (ESI) Calcd for $C_{24}H_{29}N_2O_6$ ([M+H]$^+$) 441.2020, found: 441.1857.

*(1-(3,8-Bis(methoxymethoxy)-1,2,3,4-tetrahydronaphthalen-2-yl)piperidin-4-yl)(4-fluorophenyl)-methanone ((±)-**8**):* To a solution of compound (±)-**1** (37 mg, 0.1 mmol) in CH_2Cl_2 (6 mL) was added $^{i}Pr_2NEt$ (129 g, 1 mmol). The solution was cooled to 0 °C and chloromethyl methyl ether (68 mg, 0.8 mmol) was added dropwise. The reaction was warmed to room temperature after completion of the addition and stirred overnight. The solution was quenched with water and extracted with CH_2Cl_2. The combined organic phase was washed with saturated aqueous $NaHCO_3$, saturated sodium chloride solution, and dried with anhydrous sodium sulfate. The concentrated residue was subjected to silica gel chromatography using CH_2Cl_2/EtOAc (1/1) as the eluent to afford compound (±)-**8** (25 mg, 55% yield) as a brown liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (dd, J = 8.4, 5.5 Hz, 2H), 7.16 - 7.07 (m, 3H), 6.90 (d, J = 8.2 Hz, 1H), 6.76 (d, J = 7.6 Hz, 1H), 5.22 (s, 2H), 4.83 (dd, J = 19.5, 6.9 Hz, 2H), 4.08 – 3.91 (m, 1H), 3.50 (s, 3H), 3.46 (s, 3H), 3.23 (dd, J = 16.0, 5.0 Hz, 2H), 3.10 – 2.97 (m, 4H), 2.87 (dd, J = 15.9, 9.0 Hz, 1H), 2.79 – 2.57 (m, 3H), 1.93 – 1.77 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.1, 165.6 (J = 255.5 Hz), 154.6, 135.9, 132.4, 130.8 (J = 9.1 Hz), 126.6, 124.8, 121.9, 115.7 (J = 21.2 Hz), 111.0, 95.7, 94.3, 73.4, 64.3, 56.0, 55.8, 50.0, 47.7, 44.2, 36.2, 29.5, 29.2, 22.5. HRMS (ESI) Calcd for $C_{26}H_{33}FNO_5$ ([M+H]$^+$) 458.2337, found: 458.2176.

*(-)-(1-(3,8-Dihydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)-piperidin-4-yl)(4-fluorophenyl)methanone ((-)-**1**):* Compound (-)-**1** was synthesized and chirally resolved according to previously publication as a white solid.[23] Mp 226 – 227 °C. $[\alpha]_D^{20}$ = -62.5° (1.2 mg/mL in EtOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 – 7.79 (m, 2H), 7.06 (t, J = 8.6 Hz, 2H), 6.93 (t, J = 7.8 Hz, 1H), 6.65 – 6.49 (m, 2H), 3.81 (td, J = 10.4, 5.7 Hz, 1H), 3.33 – 3.11 (m, 2H), 3.11 – 2.63 (m, 6H), 2.63 – 2.32 (m, 2H), 1.95 – 1.62 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.2, 167.0, 164.4, 153.9, 135.5, 132.3, 132.2, 130.9, 130.9, 126.9, 121.7, 121.1, 116.0, 115.7, 112.1, 77.3, 77.0, 76.7, 66.5, 65.5, 51.8, 44.6, 43.7, 37.8, 29.5, 29.1, 20.1. Anal. Calcd for $C_{22}H_{24}FNO_3$: C 71.53, H 6.55, N 3.79; found C 71.38, H 6.59, N 3.75. HRMS (ESI) Calcd for $C_{44}H_{48}F_2N_2NaO_6$ ([2M+Na]$^+$) 761.3373, found: 761.3380.

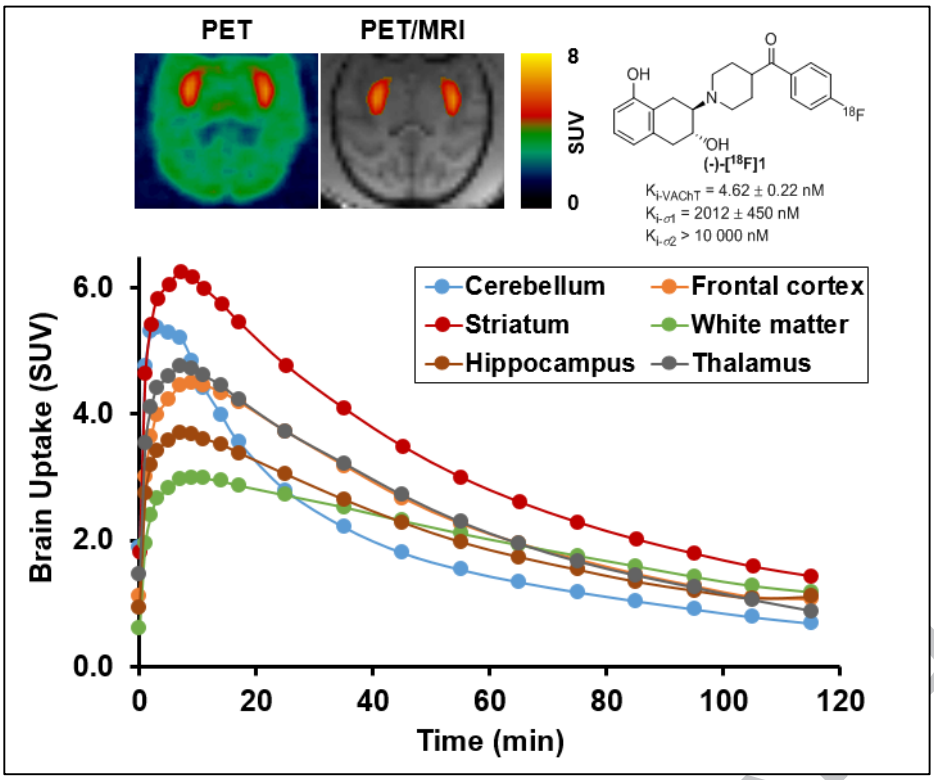
(B) VAChT and sigma receptors binding affinity studies: Binding affinities of the racemic and optically pure compounds towards VAChT, σ receptors were conducted according to published procedures.[20] Nonspecific binding for VAChT was assayed from samples that contained 1 μM of nonradioactive (±)-vesamicol. All compounds were independently assayed at least two times.

(C) Radiochemistry: A solution of ~7.0 GBq mCi K[^{18}F]F in a 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 ethanol (2 × 0.4 mL). 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. 1 - 2 mg of the corresponding precursor **(-)-6** was dissolved in DMF (300 μL) and the solution was transferred into the reaction vessel containing dried [^{18}F]fluoride/Kryptofix 2.2.2/K_2CO_3. The reaction mixture was briefly mixed and then subjected to microwave at 50 w for 120 s. After cooling down, the same procedure was repeated twice. 6 M aqueous hydrochloric acid (300 μL) was added to the mixture and the reaction was subjected to microwave at 50 w for 120 s for twice. The reaction was quenched with 3.0 mL of HPLC mobile phase (acetonitrile/0.1 M ammonium formate buffer, 23/77, v/v, pH ~6.5) and passed through an alumina cartridge. The crude product was then loaded onto an Agilent semi-preparative HPLC column (Zorbax SB-C18 250 × 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 23% acetonitrile in 77% 0.1 M ammonium formate buffer (pH ~6.5) and a flow rate at 4.0 mL/min. The HPLC fraction of the product was collected into a vial prefilled with ~50 mL water and the diluted mixture was passed through a C-18 Sep-Pak Plus cartridge to trap the product. Then the Sep-Pak was rinsed with another 20 mL water. The retained 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 NHP scans. The retention time for the product was 38 - 41 min. For quality control, an aliquot of the dose was assayed by an analytical HPLC system (Zorbax SB-C18 250 × 4.6 mm) with a detection wavelength set at 254 nm and a mobile phase consisting of acetonitrile/0.1 M ammonium formate buffer (40/60, v/v, pH 4.5). Using these conditions the retention time for (-)-[^{18}F]**1** was approximately 5.6 min at a flow rate of 1.2 mL/min. The sample was authenticated by co-injecting with the nonradiolabeled standard reference solution. The labeling yield was 24 ± 6% (n = 3) with a molar activity of 37GBq/μmol (decay corrected to the end of synthesis). The radiochemical purity was >99% and the chemical purity was >95%.

(D) *LogD measurement:* the distribution coefficient was measured by following our previous published method. [22]

(E) *MicroPET studies in an NHP and image analysis:* All the experiments conducted in NHP were in compliance with the Guidelines for the Care and Use of Research Animals under protocols approved by Institutional Animal Care and Use Committee (IACUC) at the Washington University School of Medicine. The PET scan was performed on an adult male M fascicularis (7.9 kg) with a microPET Focus 220 scanner (Concorde/CTI/Siemens Microsystems, Knoxville, TN). The procedures for NHP scan and image analysis were conducted according to our published methods. [22,20 ,23, 26]

33. Efange SM, Khare AB, von Hohenberg K, Mach RH, Parsons SM, Tu Z. *J. Med. Chem.* 2010;53:2825.



Highlights

- An enantiomeric VAChT compound with high binding affinity was synthesized.
- Radiosynthesis of the potent enantiomer was achieved in a good yield.
- The radiotracer specifically accumulated in the VAChT-enriched striatum with quick washout kinetics.