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Synthesis and in vivo evaluation of [^{11}C]MPTQ: A potential PET tracer for alpha2A-adrenergic receptors

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ABSTRACT

Radiosynthesis and in vivo evaluation of [*N*-methyl-^{11}C] 5-methyl-3-[4-(3-phenylallyl)-piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroisoxazolo[4,3-*c*]quinoline (**1**), a potential PET tracer for alpha2-adrenergic receptors is described. Syntheses of nonradioactive standard **1** and corresponding desmethyl precursor **2** were achieved from 2-aminobenzaldehyde in 40% and 65% yields, respectively. Methylation using [^{11}C]CH_3I in presence of aqueous potassium hydroxide in DMSO afforded [^{11}C]**1** in 25% yield (EOS) with >99% chemical and radiochemical purities with a specific activity ranged from 3–4 Ci/μmol (n = 6). The total synthesis time was 30 min from EOB. PET studies in anesthetized baboon show that [^{11}C]**1** penetrates BBB and accumulates in alpha2A-AR enriched brain areas.

Adrenergic receptors or adrenoceptors are G-protein coupled receptors (GPCR) that mediate the central and peripheral actions of the endogenous adrenergic amines, adrenaline and noradrenaline. Abnormalities in adrenaline and noradrenaline function have been implicated in anxiety, depression and degenerative disorders.[1–5] Based on the amino acid sequences, signaling mechanism and pharmacologic properties, these receptors are divided into three major types: alpha1, alpha2, and beta receptors.[6–9] Alpha2-adrenergic receptors are autoreceptors on noradrenergic neurons (alpha2-ARs) and are localized in both central and peripheral tissues. They have been implicated in a number of different central and peripheral pathologies. Many studies have also postulated the role of alpha2-AR in depression, mood disorder, stimulant withdrawal, social stress, alcoholic suicides, Parkinson's disease, Alzheimer's disease, and attention deficit hyper activity syndrome.[2,10–12] Alpha2-AR consists of three subtypes, alpha2A, alpha2B, and alpha2C, respectively. Postmortem human studies show that alpha2A-AR subtype is abundant in most of the brain regions with a B_{max} ranging from 34 to 90 fmol/mg of protein, whereas, only small population of alpha2B/2C was detected (8 and 5 fmol per mg of protein, respectively).[13,14] The concentration of alpha2B and 2C receptors are too small in brain to detect by PET. These studies also indicate that in the human brain the predominant and functionally relevant alpha2 receptor subtype is alpha2A-AR and its expression is not heterogeneous in brain.[14]

Several PET ligands for this receptor have been synthesized and evaluated in animals and human (Fig. 1) with limited promise. Among these [^{11}C]MK-912 and [^{11}C]WY-26703 have been evaluated in rodents and monkeys and the tracers show a fast washout of radioactivity without significant retention in alpha2-AR enriched brain regions.[15,16] Similarly, [^{18}F]fluoroatipamezole was evaluated in rat,[17] [*N*-methyl-^{11}C]mianserine in swine,[18] and [*O*-methyl-^{11}C]RS-15385-197 in rat and human.[19] However, these ligands did not show the potential to be used as a PET ligand for routine use to quantify alpha2-AR due to limited degree of regional specificity and poor heterogeneity in biodistribution of radioactivity. The most recently developed candidate, [^{11}C]R107474, has been tested in rats and showed poor in vivo specificity and hence will not be useful for the quantification of alpha2-AR.[20] [^{18}F]-RS-15385-FP, a fluoropropyl analogue of RS-16385197 has been evaluated in rats and monkeys, however, additional studies are required to prove the usefulness of this ligand to image alpha2A-AR.[21] Mirtazapine, the antidepressant drug, has been labeled and evaluated in animals and human to study the actions of antidepressant drugs in vivo. However, it is non-selective for alpha2-AR[22] and hence cannot be used to quantify this receptor.[23,24] Hence currently there is no adequate PET tracer to quantify alpha2A-AR and monitor its changes in vivo.

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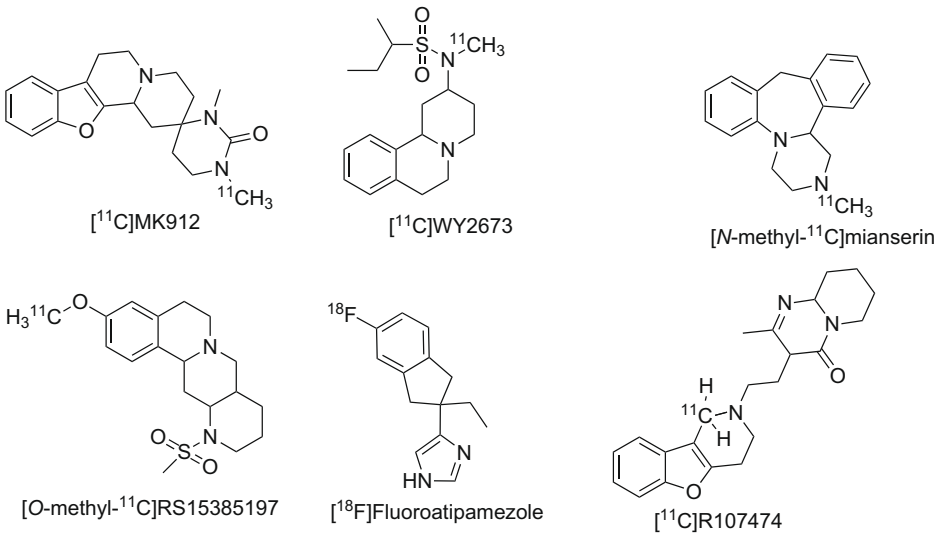
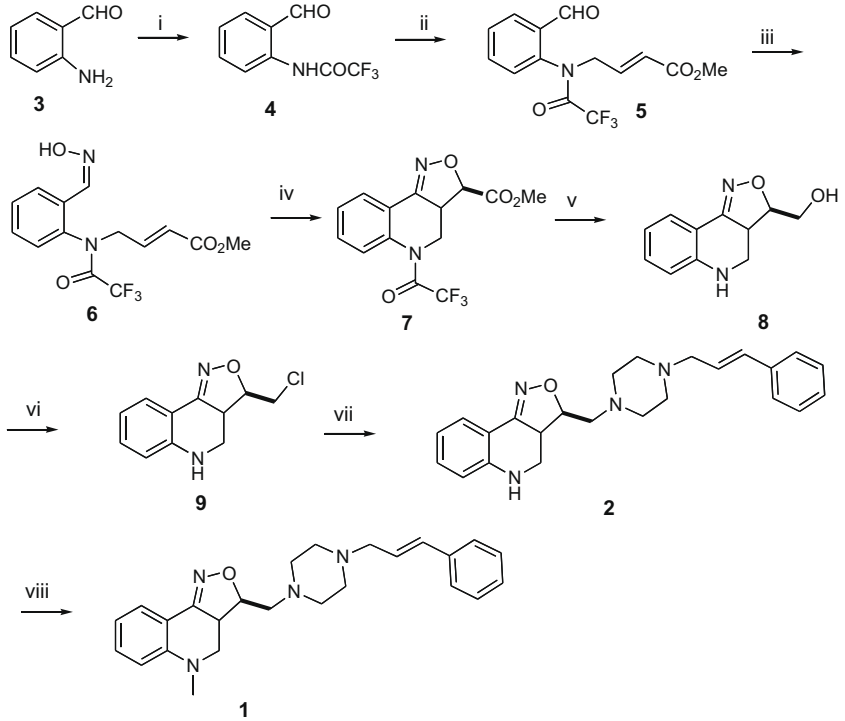


Figure 1. Alpha2A-AR CNS imaging agents.

We have selected the piperazine compound **1** as a candidate PET ligand for alpha2A-AR imaging owing to its nanomolar affinity, good selectivity and demonstrated alpha2AR blocking activity in vivo indicating CNS penetration.[25,26] Compound **1** is subtype selective towards the predominant subtype alpha2A-AR (alpha2A K_i = 1.6 nM, alpha2C K_i = 4.5 nM; 5-HTT K_i = 16 nM) as evident from competition binding studies with [^{3}H]rauwolscine and [^{3}H]paroxetine, respectively.[25] Compound **1** is 10-fold selective to alpha2A-AR over 5-HTT. In addition the lipophilicity was adequate for BBB penetration (*Clog P* value 2.86 ± 0.78 measured with ACD log P_{DB} program). As indicated above alpha2B/2C-ARs are less abundant in brain and therefore we anticipate little or no interference of these receptors in the binding of compound **1** in tracer dose to alpha2A-AR.[14] Since the B_{max} of alpha2A-AR and 5-HTT are comparable in brain,[14,27] we anticipate a 10-fold affinity of compound **1** to alpha2A-AR over 5-HTT is advantageous as an imaging agent with PET. Additionally, the availability of suitable sites for incorporating C-11 isotope encouraged us to test compound **1** for its ability to image alpha2A-AR in vivo by PET. Synthesis of nonradioactive standard **1** and desmethyl precursor **2** were achieved in moderate yield starting from 2-amino benzaldehyde **3** (25, Scheme 1). The trifluoroacetyl compound **4** was obtained from 2-aminobenzaldehyde **3** by treating it with trifluoroacetic anhydride in presence of triethyl amine, which was then condensed with methyl-4-bromocrotonate to afford ester **5**. Compound **5** was subsequently treated with hydroxylamine



Scheme 1. Syntheses of compounds **1** and **2**. Reagents and conditions: (i) TFAA, Et_3N, ether, −10 to 0 °C, 96%; (ii) methyl-4-bromocrotonate, NaH, 18-crown-6, DMF, 60%; (iii) $NH_2OH{\cdot}HCl$, pyridine, ethanol, 65%; (iv) chloramine-T, ethanol, 48%; (v) $NaBH_4$, THF/H_2O, 90%; (vi) CCl_4, Ph_3P, THF, 62%; (vii) 1-cinnamylpiperazine, KI, dioxane, 65%; (viii) HCHO, $NaCNBH_3$, $ZnBr_2$, CH_3OH 40%.

hydrochloride and chloramine-T, respectively, to obtain the isoxazole **7**. Ester group in **7** was reduced to obtain alcohol **8,** which was then converted to the chloride **9** in moderate yield using carbon tetrachloride and triphenylphosphine. The chloride **9** was condensed with 1-cinnamyl piperazine to obtain desmethyl precursor **2** in 65% yield. The nonradioactive standard **1** was obtained by methylation of amine **2** using formaldehyde and sodium cyanoborohydride (40%).

Radiolabeling was initially attempted by treating precursor **2** in DMF (0.5 mg in 0.5 mL) with [^{11}C]CH_3I using potassium *tert*-butoxide and heating at 80 °C. The yield of [^{11}C]**1** obtained was 30–40 mCi (10% yield at EOS) with specific activity in the range of 2–2.4 Ci/μmol (n = 4). However, reaction of precursor **2** with aqueous KOH and [^{11}C]CH_3I in DMSO (Scheme 2) resulted in better radiochemical yield (25% at EOS, n = 6). Therefore, we adopted the later experimental condition for the production of [^{11}C]**1** for further studies. Chemical and radiochemical purities obtained were >99% and the improved specific activity was 3–4 Ci/μmol.[28] The partition coefficient for [^{11}C]**1** obtained by standard shake flask method is 2.5.[29]

To determine the BBB permeability and distribution of [^{11}C]**1**, we conducted baboon PET scanning experiments adopting our established procedures.[30] Anesthesia (isoflurane 1.5–2.0%) was induced in a fasted baboon (male, 5 year) immobilized with ketamine (10 mg/kg, im) via endotracheal tube. After a 10 min transmission scan, 4.43 mCi of [^{11}C]**1** (specific activity 3 Ci/μmol) was injected as an iv bolus and emission data were collected for 10 min in 3–D mode in a Siemens ECAT EXACT HR+ camera (CPS/Knoxville, TN). Figure 2, the sum of last 60 min images, shows that [^{11}C]**1** enter the BBB and the radioactivity is retained in brain with homogeneous distribution. The time activity curves (TACs) demonstrate the uptake is consistent with the known distribution of alpha2A-AR, the predominant subtype of alpha2-AR (Fig. 3).[14] Metabolite analyses show 35 ± 5% and 21 ± 3% of unmetabolized [^{11}C]**1** at 30 and 90 min, respectively. The radioligand exhibits negligible protein binding in human and baboon plasma. The radioactivity distribution is almost homogeneous except for thalamus, amygdala, insular cortex, and hippocampus.

The target to cerebellum ratios for thalamus, amygdala, caudate, putamen, hippocampus, cingulate cortex, temporal cortex, and occipital cortex are 1.66, 166, 1.4, 1.52, 1.34, 1.28, and 0.9, respectively.[14] This distribution pattern is not in agreement with the reported 5-HTT distributions and this rules out the possibility of [^{11}C]**1** binding to 5-HTT for which it has 16 nM affinity.[31–33] The bindings in occipital cortex of four selective SERT tracers in non-human primates are higher than in cerebellum unlike

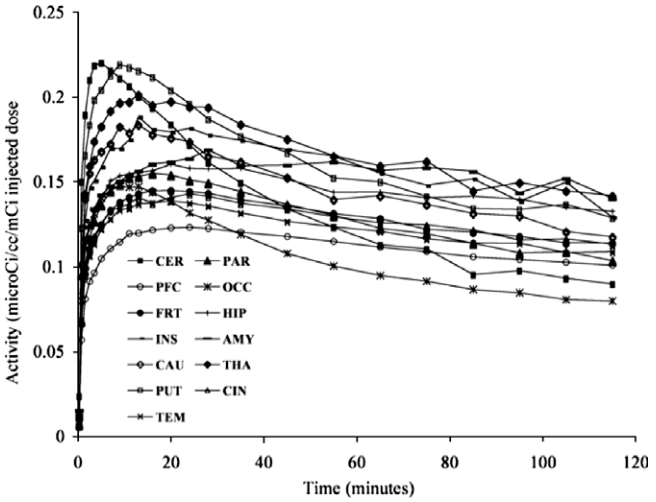
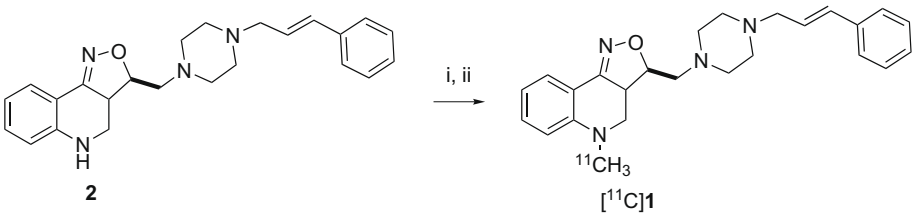


Figure 3. Time activity curves of [^{11}C]**1** in baboon. AMY = Amygdala; CAU = Caudate; CER = Cerebellum; HIP = Hippocampus; INS = Insula; FRT = Frontal cortex; OCC = Occipital cortex; PAR = Parietal cortex; PFC = Prefrontal cortex; THA = Thalamus, TEM = temporal cortex; CIN = Cingulate cortex; PUT = Putamen.



Scheme 2. Radiosynthesis of [^{11}C]**1**. Reagent and conditions: (i) KOH, DMSO, $^{11}CH_3$I, 5 min; (ii) HPLC purification, 25% (EOS).

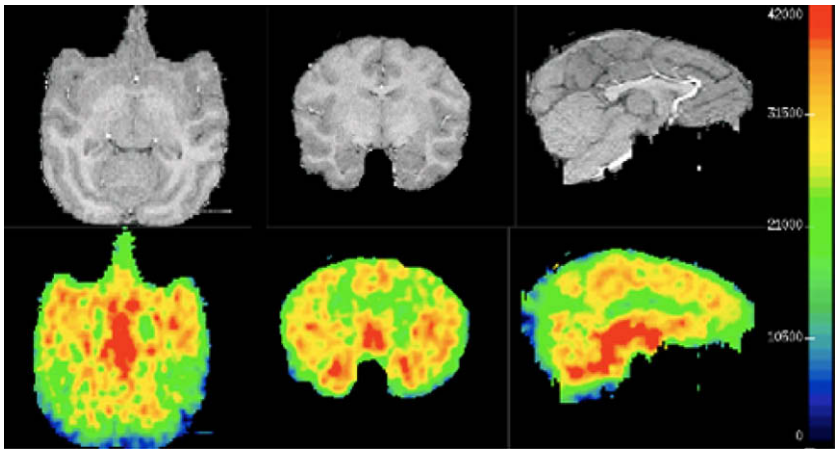


Figure 2. PET and MRI images of [^{11}C]**1** in baboon. First row: MRI; second row: Baseline scan First column: axial, middle column: coronal, last column: sagittal views.

[^{11}C]**1**.[31] Also another discrepancy of SERT ligands compared to [^{11}C]**1** is their higher binding in hippocampus, compared to striatum (caudate and putamen) and higher binding in temporal cortex than cingular cortex.[31] These findings further rule out the 5-HTT binding of [^{11}C]**1** in baboon brain.[30]

In summary, we successfully synthesized [^{11}C]**1**, a PET tracer agent for alpha2A-AR. The total time required for the radiosynthesis was 30 min from EOB using [^{11}C]methyl iodide in DMSO. [^{11}C]**1** was obtained in 25% yield (EOS) with >99% chemical and radiochemical purities and the specific activity ranged from 3–4 Ci/μmol ($n = 6$). PET studies in anesthetized baboon show that [^{11}C]**1** penetrates BBB and accumulates in brain areas enriched with alpha2A-AR. Experiments are under progress to further quantify alpha2A-AR with [^{11}C]**1**.

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28. *Radiosynthesis of* [^{11}C]**1**: The precursor desmethyl-1 (0.5–1.0 mg) was dissolved in 500 μL of DMSO in a capped 5 mL V-vial containing potassium hydroxide (1 mg) and the resultant solution was allowed to stand for 2 min. High specific active [^{11}C]CO_2 produced from RDS112 Cyclotron (~37 GBq) was subsequently converted into [^{11}C]CH_3I and is transported by a stream of argon (10–20 mL/min) into the vial over approximately 5 min at room temperature. At the end of the trapping, the product mixture was diluted with 0.5 mL of acetonitrile and was directly injected into a semi preparative RP-HPLC (Phenomenex C18, 10 × 250 mm, 10) and eluted with acetonitrile/0.025 M disodium hydrogen phosphate solution (60:40) at a flow rate of 10 mL/min. The precursor eluted after 2–3 min during the HPLC analysis. The product fraction with a retention time of 7–8 min based on γ-detector was collected, diluted with 100 mL of deionized water, and passed through a classic C-18 Sep-Pak® cartridge. Reconstitution of the product in 1 mL of absolute ethanol afforded [^{11}C]**1** (25% yield, based on $^{11}CH_3I$ at EOS). A portion of the ethanol solution was analyzed by analytical RP-HPLC (Phenomenex, Prodigy ODS(3) 4.6 × 250 mm, 5; mobile phase: acetonitrile/0.025 M disodium hydrogen phosphate solution; 60:40, flow rate: 2 mL/min, retention time: 7 min, wavelength: 230 nm) to determine the specific activity and radiochemical purity. Then the final solution of the [^{11}C]**1** in 10% ethanol-saline was analyzed to confirm the chemical and radiochemical purities.
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