THE SYNTHESIS, ^{11}C-LABELING, AND BIOLOGICAL EVALUATION OF (*R*)-*N*-[^{11}C]METHYL-3-PYRROLIDYL BENZILATE AS AN ACETYLCHOLINE-SENSITIVE LIGAND FOR THE MUSCARINIC ACETYLCHOLINE RECEPTOR

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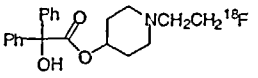
Key Words: carbon-11, muscarinic, acetylcholine, receptor, benzilate

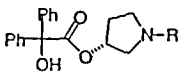
The muscarinic acetylcholine receptor (mAChR) is a G-protein coupled receptor which has been implicated in the regulation of higher cognitive functions such as learning and memory.(1) In addition, alterations in mAChR densities and loss of choline acetyltransferase activity are well-known trademarks of Alzheimer's disease.(1,2,3) These observations have led to the hypothesis that enhancement of the cholinergic system using mAChR agonists or acetylcholinesterase (AChE) inhibitors can lead to amelioration of AD symptoms. Although numerous approaches to bolstering the cholinergic system have been developed (AChE inhibitors, acetylcholine-releasing agents, and cholinergic agonists), relatively little is known on how these drugs affect receptor occupancy by acetylcholine (ACh). The *in vivo* imaging of mAChR availability, using PET, might provide a method for evaluating new or existing pharmaceutical approaches to enhancing the cholinergic system, provided a suitable radiopharmaceutical with demonstrated sensitivity to ACh levels were to be available.

Towards this goal, we had previously reported the synthesis, ^{18}F-labeling, and *in vitro* K_i values of of *N*-(2-[^{18}F]fluoroethyl)-4-piperidyl benzilate (1), a moderate affinity (K_i = 1.7 nM), non-subtype selective antagonist. This compound was shown to have good uptake in muscarinic receptor-rich regions of the rodent brain, was sensitive to changes in endogenous ACh levels, and displayed excellent bolus pharmacokinetics in primates.(4,5) However, difficulties were encountered when applying this compound in a bolus plus infusion protocol in primates. As a result, we turned our efforts towards the synthesis and biological evaluation of (*R*)-*N*-[^{11}C]methyl-3-pyrrolidyl benzilate (2a, K_i = 0.72 nM) as a candidate for an ACh-sensitive PET ligand for mAChRs.

Compound **2a** was prepared by reacting the precursor (**2b**) with [^{11}C]methyl triflate in dimethylacetamide at room temperature. After diluting with cyclohexane, the reaction contents are loaded onto a short

alumina column, and the excess dimethylacetamide is removed by washing with additional cyclohexane. The desired compound is then eluted with a $MeOH/CH_2Cl_2$ solution, followed by evaporation of the solvent, and reformulation in ethanol/saline. The entirely automated reaction sequence takes 20 minutes following EOB. Radiochemical purities are typically >95%, with no evidence of the biologically active **2b**. Radiochemical yields are typically 350-400 mCi for a 25 min, 20 μamp beam.





2a: R = $^{11}CH_3$
2b: R = H

Initial studies have shown that **2a** has excellent retention in the mAChR-rich regions of the rodent brain. Moreover, the striatum/cerebellum, cortex/cerebellum, and striatum/cortex ratios for **2a** exceed those of **1** for both the mouse and rat. Bolus studies in monkeys showed that **2a** had pharmacokinetics superior to those of other known ^{11}C-labeled muscarinic ligands, such as [^{11}C]TRB and [^{11}C]NMPB. In contrast to **1**, bolus plus infusion experiments with **2a** in monkeys have shown that it is possible to achieve cortical equilibrium within short time frames. We are currently investigating the sensitivity of **2a** to phenserine-induced ACh changes.

In conclusion, we have synthesized a novel carbon-11 labeled muscarinic antagonist for use as an ACh-sensitive ligand for the mAChR system. The radiochemical synthesis of **2a** is remarkably easy and reproducible, requires no HPLC purification, and provides excellent radiochemical yields within a short overall reaction time. The biological data for **2a** suggests that this compound is superior to many other known muscarinic PET radioligands in terms of its retention in mAChR-rich regions and pharmacokinetics in rodents and primates.

References

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Acknowledgments

We are grateful for support of this research through grants from the Department of Energy (DE-FG02-ER60561) and the National Institutes of Health (T-32-CA09015).