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Synthesis and biological evaluation of positron emission tomography radiotracers targeting serotonin 4 receptors in brain: [^{18}F]MNI-698 and [^{18}F]MNI-699

Fabien Caillé, Thomas J. Morley, Adriana Alexandre S. Tavares, Caroline Papin, Nicole M. Twardy, David Alagille, H. Sharon Lee, Ronald M. Baldwin, John P. Seibyl, Olivier Barret, Gilles D. Tamagnan *

Molecular NeuroImaging LLC, New Haven, CT 06510, USA
Institute for Neurodegenerative Disorders, New Haven, CT 06510, USA

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ABSTRACT

Two new benzodioxane derivatives were synthesized as candidates to image the serotonin 4 receptors by positron emission tomography (PET) and radiolabeled with fluorine-18 via a two-step procedure. Competition binding assays demonstrated that MNI-698 and MNI-699 had sub-nanomolar binding affinities against rat striatal 5-HT$_4$ receptors (K_i of 0.20 and 0.07 nM, respectively). PET imaging in rhesus monkey showed that the regional brain distribution of [^{18}F]MNI-698 and [^{18}F]MNI-699 were consistent with the known densities of 5-HT$_4$ in brain. [^{18}F]MNI-698 and [^{18}F]MNI-699 are among the first fluorine-18 radiotracers developed for imaging the 5-HT$_4$ receptors in vivo and are currently under preclinical investigation in primates for future human use.

Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter known to interact with different receptors and transporters in the brain. Dysregulation of the serotonergic system has been implicated in multiple neuropsychiatric and neurodegenerative disorders.[1] The serotonin 4 (5-HT$_4$) receptor is a member of the 7-membrane spanning G-protein coupled receptors positively linked to adenylate cyclase and has been detected in the brain of mammalian species, including rodents, non-human primates and humans.[2–4] In the central nervous system, 5-HT$_4$ receptors are primarily localized in the limbic and nigrostriatal regions, which are associated with learning and memory functions.[5] Preclinical studies using animal models of Alzheimer's disease (AD) have shown that administration of 5-HT$_4$ agonists increased acetylcholine release, resulting in cognitive improvement on both short and long-term memory.[5] Moreover, post-mortem studies using brain tissue from AD patients have shown a decrease in 5-HT$_4$ receptors density in these patients' hippocampus compared with control subjects.[6–8] The 5-HT$_4$ receptors have also been implicated in other neuropsychiatric disorders such as anxiety and depression.[9,10] Non-invasive imaging of 5-HT$_4$ using either positron emission tomography (PET) or single photon emission computed tomography (SPECT) is extremely useful for studies evaluating new drugs targeting 5-HT$_4$ receptors and also for investigations of pathophysiological changes in 5-HT$_4$ receptor density in a variety of neuropsychiatric and neurodegenerative disorders.

Among the compounds developed to target the 5-HT$_4$ receptor, benzodioxane derivatives, such as SB 204070 (**1**, Fig. 1), have been shown to be potent and selective antagonists.[11] The iodo analogue, SB 204710 (**2**, Fig. 1), showed a high affinity for 5-HT$_4$ receptors and after labeling with iodine-123 for SPECT imaging was found to rapidly (maximum uptake less than 20 min, 2.3% injected dose) accumulate in 5-HT$_4$ rich regions in non-human primate brain.[12,13] Other analogues of compound **1** have been labeled for PET imaging, [^{11}C]SB 207145 ([^{11}C]**3**, Scheme 1) has been evaluated in animal and human.[14–16] Although imaging data obtained using [^{11}C]SB 207145 demonstrated its suitability for imaging 5-HT$_4$ receptors in brain, we sought to develop tracers incorporating the longer lived isotope fluorine-18 (^{18}F $t_{1/2}$ = 109.7 min; ^{11}C $t_{1/2}$ = 20.3 min) to be able to perform large scale imaging studies. Recently, Pike et al. reported the first fluorine-18 radiofluorinated 5-HT$_4$ radiotracer by radiofluoromethylation of an analogue of **1**.[17] However, this radiotracer displayed a low binding affinity towards recombinant human 5-HT$_4$ receptor (K_i = 17 nM) and was not further investigated.[17] Herein we report the synthesis, in vitro binding affinity, fluorine-18 labeling, and the first in vivo evaluation in non-human primate of [^{18}F]MNI-698 and [^{18}F]MNI-699 (Scheme 2).

Given that compounds **2** and **3** display a high affinity and selectivity for 5-HT$_4$ receptors, and were found to be suitable agents for

* Corresponding author. Tel.: +1 203 401 4309; fax: +1 203 789 2119.
E-mail address: gtamagnan@mnimaging.com (G.D. Tamagnan).

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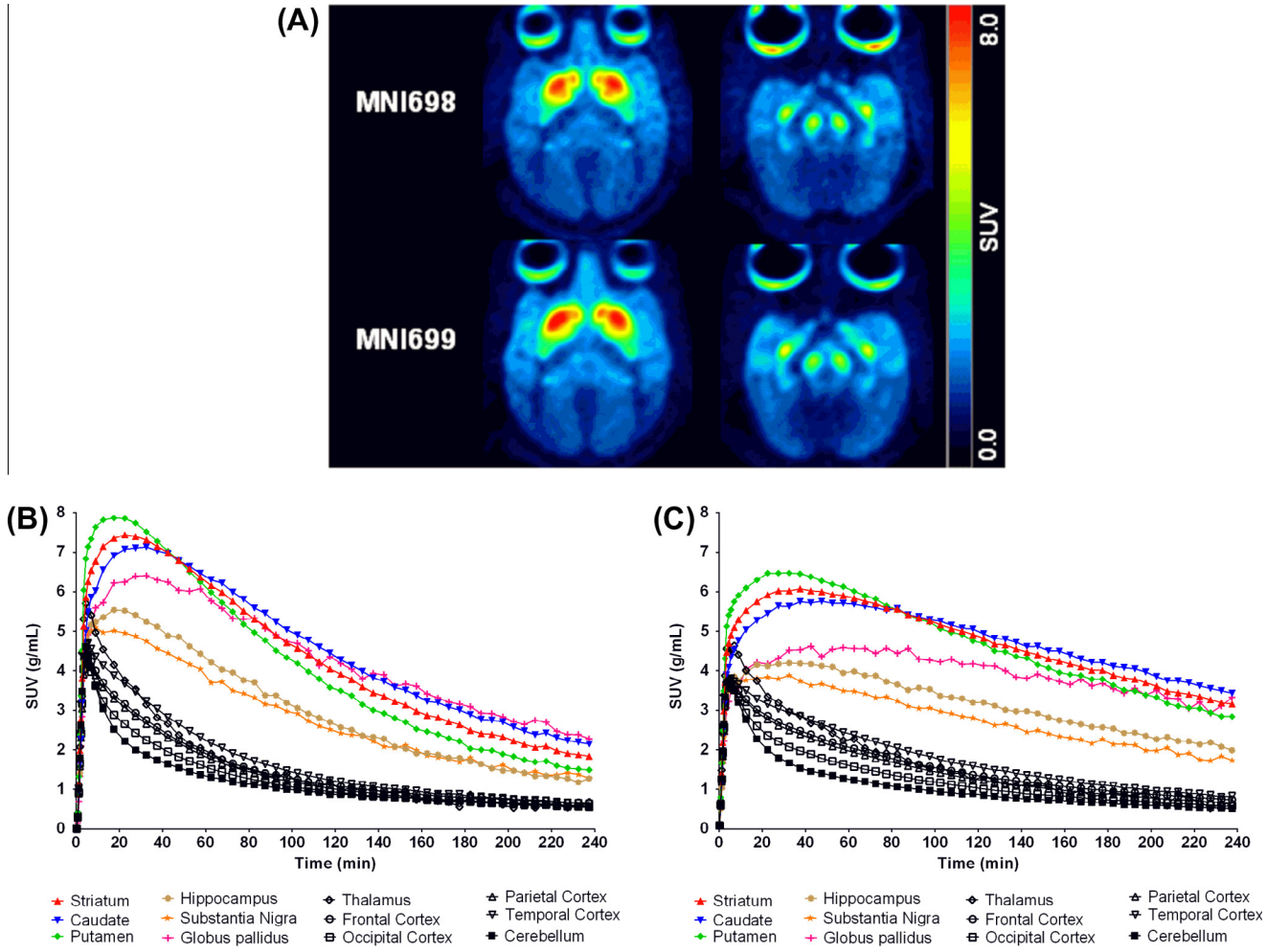
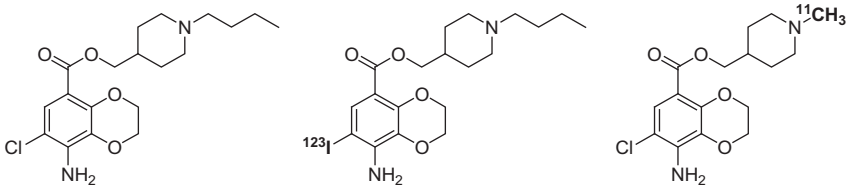


Figure 1. (Top) Representative PET SUV images (sum from 0 to 240 min pi) obtained following a single bolus intravenous injection of [^{18}F]MNI-698 or [^{18}F]MNI-699 in a female rhesus monkey. (Bottom) SUV time-activity curves obtained following intravenous bolus injection of [^{18}F]MNI-698 (A) and [^{18}F]MNI-699 (B). Note the high uptake in 5-HT$_4$ rich regions, such as striatum, caudate, putamen, globus pallidus, hippocampus and substantia nigra, compared to lower uptake in regions with low levels of 5-HT$_4$.

imaging of those receptors in vivo, we chose fluorinated analogues that were structurally similar to these molecules, whereby the fluorine is incorporated at the terminal end of the *N*-alkyl chain of the piperidine moiety. The synthesis of these compounds is presented in Scheme 2, starting with benzodioxane derivative **4**,[18] which was selectively chlorinated by treatment with N-chlorosuccinimide in DMF to afford compound **5**. Transesterification of *tert*-butyl 4-(hydroxymethyl)piperidine-1-carboxylate with compound 5 gave ester 6, which, after removal of the *N*-Boc group, afforded the free amine **7**. MNI-698 and MNI-699 were obtained by N-alkylation with 2-fluoroethyl 4-methylbenzenesulfonate[19] and 2-fluoropropyl 4-methylbenzenesulfonate,[20] respectively. This reaction was slow and no more than 50% conversion was obtained after 18 hours at reflux in acetonitrile with 1.5 equiv of fluoroalkyl tosylate. Interestingly, the aniline moiety in position 8 did not need protection during the synthesis, in contrast to previous reports.[10,17] Unfortunately, attempts to synthesize primary tosylate **8**, which could be used as precursor for radiolabeling by nucleophilic substitution with [^{18}F]fluoride, were unsuccessful, owing to degradation during purification. Therefore, a two-step radiolabeling procedure using amine **7** as precursor was tested.

Radiolabeled [^{18}F]MNI-698 and [^{18}F]MNI-699 were prepared using a GE TRACERlab™ FX$_{F-N}$ automated synthesizer, by



SB 204070 (**1**) [^{123}I]SB 207710 ([^{123}I]**2**) [^{11}C]SB 207145 ([^{11}C]**3**)

Scheme 1. Structure of SB 204070 and analogous radiotracers for imaging of 5-HT$_4$ receptors.