ACS Chemical Neuroscience

Letter

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Synthesis and Evaluation of Methylated Arylazepine Compounds for PET Imaging of 5-HT_{2c} Receptors

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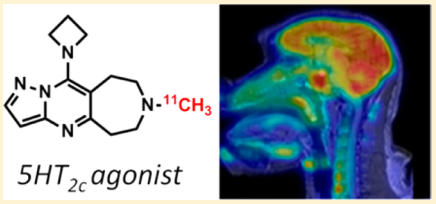
Supporting Information

ABSTRACT: The serotonin 5-HT_{2c} receptor is implicated in a number of diseases including obesity, depression, anxiety, and schizophrenia. In order to ascribe the role of 5-HT_{2c} in these diseases, a method for measuring 5-HT_{2c} density and function in vivo, such as with positron emission tomography (PET), must be developed. Many high-affinity and relatively selective ligands exist for 5-HT_{2c} but cannot be accessed with current radiosynthetic methods for use as PET radiotracers. We propose that *N*-methylation of an arylazepine moiety, a frequent structural feature in 5-HT_{2c} ligands, may be a suitable method for producing new radiotracers for 5-HT_{2c}. The impact of *N*-methylation has not been previously reported. For the agonists that we selected herein, *N*-methylation was found to increase affinity up to 8-fold without impairing selectivity. Compound 5, an *N*-methylated azetidine-derived arylazepine, was found to be brain penetrant and reached a brain/blood ratio of 2.05:1. However, our initial test compound was rapidly metabolized within 20 min of administration and exhibited high nonspecific binding. *N*-Methylation, with 16 ± 3% isolated radiochemical yield (decay corrected), is robust and may facilitate screening other 5-HT_{2c} ligands as radiotracers for PET.



KEYWORDS: *Serotonin, PET imaging, carbon-11, 5-HT_{2c} agonist*

Serotonin (5-hydroxytryptamine, 5-HT) is directly involved in regulating behavioral and visceral functions in all animals, and dysregulation may lead to a large spectrum of psychiatric disorders. Presently, there are limited methods to individually study the contributions to disease of one receptor among the 14 5-HT subtypes that have been described. The 5-HT_2 family consists of 5-HT_{2a}, an important excitatory receptor and frequent drug target; 5-HT_{2b}, which is found almost exclusively in the periphery and linked to valvulopathies; and 5-HT_{2c}, which is linked to numerous and diverse brain disorders and yet has received less attention in psychopharmacology and neuroimaging than its homologue, 5-HT_{2a}. The 5-HT_{2a} receptor is implicated in the pathogenesis of major depressive disorder and bipolar disorder, and studies of 5-HT_{2a} benefit from the existence of highly selective radioligands (most importantly, [^{18}F]Altanserin) that facilitate the discovery of connections between receptor dysfunction and disease. A large body of research suggests that dysfunction of 5-HT_{2c} is related to depression, schizophrenia, drug abuse, Parkinson's disease, anxiety, and obesity.[1−8] In fact, the first FDA approval of a 5-HT_{2c} target molecule, lorcaserin, occurred in June 2012. However, direct links between these diseases and 5-HT_{2c} receptor abnormalities have been difficult to elucidate due to the lack of a method for determining 5-HT_{2c} receptor concentration in vivo.

Autoradiographic and immunohistochemical methods have provided information about 5-HT_{2c} receptor density in animal models of disease, but the ability to fully ascribe the role of 5-HT_{2c} in disease is limited by the inability to study the unperturbed system in vivo, ideally in humans.[9,10] Over the past decade, there have been major advances in the ability to visualize serotonin receptors using noninvasive imaging;[11] however, to date, there are no radiotracers for visualization of the 5-HT_{2c} receptor in vivo, and the tools available for in vitro autoradiography are nonselective. The 5-HT_{2c} receptor (previously known as the 5-HT_{1c} receptor) is abundantly expressed in multiple brain regions such as the choroid plexus, hippocampus, cortex, and amygdalae, with some reports documenting a density of 800−1600 fmol/mg.[12−16] As this density is as high as that of 5-HT_{1a} and 5-HT_{2a}, both of which have been successfully imaged using PET, and is localized in brain regions of sufficient volume to alleviate partial volume effects, it should be possible to measure 5-HT_{2c} binding in the human brain using PET.

While many excellent ligands for 5-HT_{2c} receptors now exist, the development of a radioligand has, to date, been hindered greatly by the lack of strategies for radiolabeling. Many of these compounds feature an arylazepine motif (Figure 1, in red) consisting of an azepine heterocycle fused with one or more aryl ring structures. Although considerable advances have been made in labeling nonpendant functional groups with carbon-11, no general method yet exists for carbon-11 labeling of

Received: December 4, 2012
Accepted: December 4, 2012
Published: December 4, 2012

   dx.doi.org/10.1021/cn300223d | *ACS Chem. Neurosci.* 2013, 4, 261−265

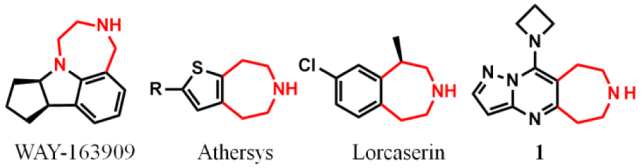


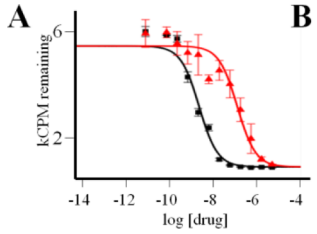
Figure 1. Examples of serotonin-2c (5-HT$_{2c}$) agonists containing an arylazepine moiety.

arylazepines.[17,18] The prevalence of arylazepines as a pharmacophore in 5-HT$_{2c}$ ligands prompted us to consider a modular approach to ligand evaluation using a simple *N*-methylation strategy for radiolabeling with carbon-11.[19] Methylation is a common method of producing radiolabeled compounds with carbon-11, and it may be possible to accelerate the process of 5-HT$_{2c}$ radiotracer discovery by assessing whether methylation is tolerated at this position.

To this end, we selected a recently published novel ligand with an excellent binding profile in vitro to probe the impact of arylazepine *N*-methylation. This ligand (**1**) exhibits high affinity for 5-HT$_{2c}$ receptors and reasonable selectivity over 5-HT$_{2a}$ and 5-HT$_{2b}$ (EC$_{50}$ for 5-HT$_{2a}$, 5-HT$_{2b}$, and 5-HT$_{2c}$ = 2348, 94, and 5.4 nM, respectively), from which we projected adequate in vivo affinity for imaging given the concentration of total binding sites (B_{max}).[20] Effects on feeding behavior in rats as compared to lorcaserin suggested compound **1** may have suitable blood-brain barrier penetration and pharmacokinetics.[21] We synthesized the methylation product of compound **1** and a related analogue and compared binding affinity and selectivity at 5-HT$_{2c}$ for the methyl and desmethyl compounds. In addition, we radiolabeled compound **1** with carbon-11 and performed PET imaging in rodents and nonhuman primates for evaluation as a PET radiotracer. This process provides a roadmap for expanding this modular approach to other arylazepine compounds in radiotracer design for 5-HT$_{2c}$.

The arylazepine scaffold was synthesized by published methods and produced chloro-precursor **2** (Scheme 1).[20] Compound **1** was obtained by reaction of **2** with azetidine. Ether derivatives **3** and **4** were synthesized for 5-HT$_{2c}$ affinity comparison and were readily obtained by alkoxide displacement of chloride **2**. Methylation of the arylazepines was accomplished by reductive amination with formaldehyde and sodium cyanoborohydride in the presence of acetic acid.

Methylated compound **8** exhibited 3-fold greater affinity for 5-HT$_{2c}$ versus that of the desmethyl analogue (Boc-deprotected **2**) and exhibited no observed interaction with 5-HT$_{1a}$, 5-HT$_{2a}$, or 5-HT$_{2b}$. Similarly, methylated **5** as compared to desmethyl **1** had greater affinity (1.3:1) with the only discernible off-target effect being increased binding at 5-HT$_3$ (2.8:1). Compounds **5** and **1** had >50% binding at 5-HT$_{2c}$, for which a secondary assay was performed (Figure 2). By radioligand competition assay



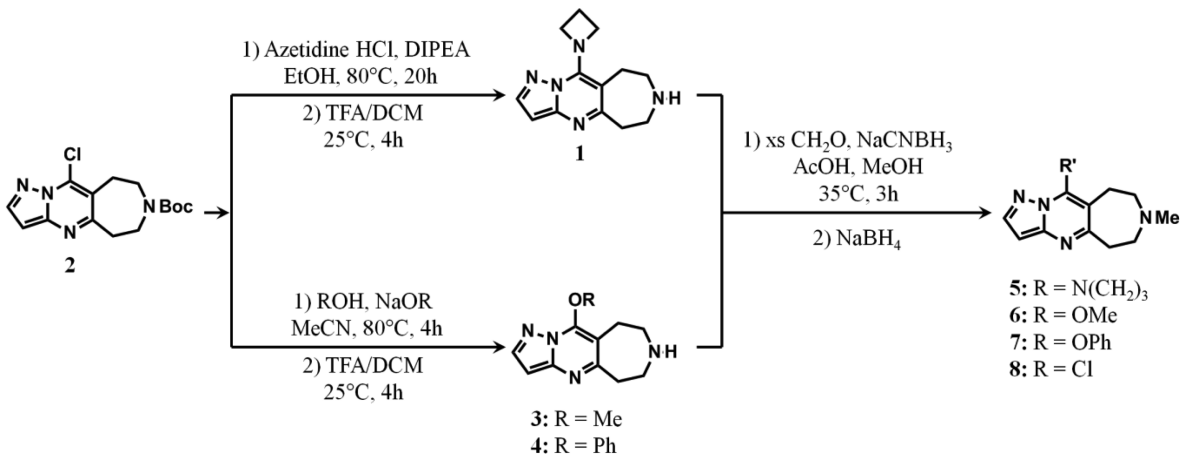
Receptor	Ki (nM)	
	2	**5**
5-HT$_{1a}$	1599	1134
5-HT$_{2b}$	1862	>10000
5-HT$_{2c}$	648	75

Figure 2. PDSP evaluation of binding affinity. (A) 5-HT$_{2c}$ binding was determined using [^{3}H]-mesulergine competition. Representative data for **5** (red) and a control, ritanserin (black), are shown. (B) K_i values determined by PDSP for **2** and **5** at high-affinity 5-HT$_{2c}$ receptors.

with [^{3}H]-mesulergine, **5** showed 8.5-fold increased binding over **1** at 5-HT$_{2c}$. Compound **7** displayed primary binding of 40.6% at 5-HT$_{2c}$. In both pairs of methylated and desmethyl compounds (e.g., **8** and Boc deprotected **2**, **5**, and **1**), *N*-methylation appeared to increase 5-HT$_{2c}$ binding by affinity assays and radioligand competition assay in **5** and **1**. This was an indication that methylation may be a viable option for the development of a 5-HT$_{2c}$ PET radiotracer. We selected **5** for carbon-11 radiolabeling because it possessed the most preferable binding profile, with both precursor **1** and **5** having a sufficient dissociation constant to support imaging of 5-HT$_{2c}$ (K_i = 648 and 75 nM, respectively, compared to B_{max} = 800–1600 fmol/mg for 5-HT$_{2c}$). Additionally, no significant binding was observed in other widely expressed G-protein coupled receptors.

Compound [^{11}C]**5** was prepared from **1** using high specific activity [^{11}C]-iodomethane with an isolated radiochemical yield of 16.5 ± 3% (n = 4) after decay correction from $^{11}CO_2$. Purification was accomplished by semipreparative HPLC in 10.5 min (Figure 3). HPLC chromatograms indicated >85% methylation efficiency. The total time of synthesis was approximately 36 min from end-of-beam to final isolation,

Scheme 1. Synthesis of 5-HT$_{2c}$ Agonists



dx.doi.org/10.1021/cn300223d | ACS Chem. Neurosci. 2013, 4, 261–265

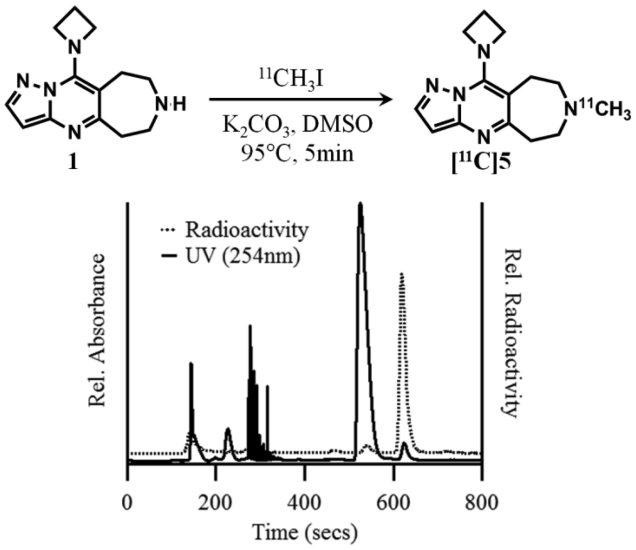


Figure 3. Synthesis and purification of carbon-11 labeled **5**. The reaction of arylazepines with $^{11}CH_3I$ at high specific activity is fast and efficient resulting in only one radiolabeled product.

producing on average 1.9 GBq of [^{11}C]**5**. The product was formulated in a 10% ethanol in saline solution for PET imaging experiments.

[^{11}C]**5** was intravenously administered to a *Papio anubis* baboon for PET imaging. [^{11}C]**5** exhibited excellent brain penetration, and we observed a sustained brain concentration that was twice the concentration in blood (2.05:1). The regional uptake of [^{11}C]**5** within the brain was markedly homogeneous and did not correlate with the known 5-HT_{2c} density.[10,12,15] Moreover, the time−activity curves for several regions of interest (Figure 4B) indicated nearly identical pharmacokinetics throughout the brain. The choroid plexus, which is reported to have the highest concentration of 5-HT_{2c} receptors, displayed low uptake that was comparable to that seen in other brain regions. The cerebellum, a region known to be bereft of 5-HT_{2c} receptors, had higher than expected uptake. Summed PET images at both early and late time points confirm uniform distribution of [^{11}C]**5** (Figure 4A). Therefore, the signal observed in the baseline scan of [^{11}C]**5** appeared to be dominated by nonspecific binding.

To test for specific binding with [^{11}C]**5**, a second imaging study was conducted in which [^{11}C]**5** was administered 10 min after ketanserin (1 mg/kg IV, K_i at 5-HT_{2c} = 161 nM), an antagonist commonly used for 5-HT_2 blockade.[21] Neither reduction in uptake nor a change in the distribution or kinetics was observed, suggesting that the PET images reflects nonspecific uptake almost exclusively (Figure 4C).

Metabolism data were derived from solid phase extraction of plasma sampled at 5, 10, 20, 30, 45, 60, and 80 min. Metabolism of [^{11}C]**5** was very rapid (Figure 5B) and began to reach the limit of detection over background at 20 min. Alongside the depletion of [^{11}C]**5** was the emergence of a single, more polar radiometabolite that represented 52% of radioactivity in plasma by 10 min. Clearance from plasma was also rapid.

To further verify that images were dominated by nonspecific binding, we evaluated [^{11}C]**5** in male Sprague−Dawley rats (n = 8). The rodents were utilized in pairs and pretreated with vehicle (n = 3), **5** (n = 3), altanserin (n = 1), and ketanserin (n =1). Each rat received intravenous pretreatment 10 min before

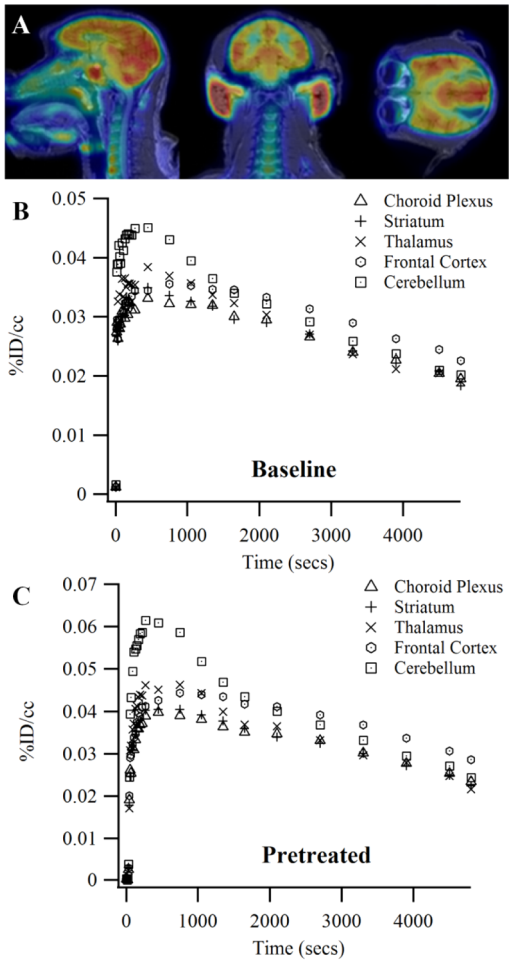


Figure 4. MR-PET analysis of [^{11}C]**5** in *Papio anubis* baboon. (A) Summed image of PET data from 1 to 21 min post injection (overlay with MR). (B) Representative time−activity curves for brain regions of interest in baseline [^{11}C]**5** study. (C) Time−activity curves for brain regions from pretreatment study with 1 mg/kg ketanserin.

injection of [^{11}C]**5**. Average uptake in the whole brain was 0.197 ± 0.05% injected dose over all treatment groups. At 90 min, the brain to blood ratio in the vehicle group (1.58:1) was similar to that seen in baboon. The organs of greatest accumulation were the liver, kidneys, and intestines at 90 min (Figure 5A), which is expected after metabolism and excretion of the radiotracer. We noted that whole brain uptake was greater with pretreatment, potentially indicating saturation of peripheral binding sites; however, these data confirmed that PET images with [^{11}C]**5** represent nonspecific binding.

Although [^{11}C]**5** does not appear to be a suitable candidate for in vivo 5-HT_{2c} imaging, the labeling and evaluation of it provides a roadmap for *N*-methylation of other arylazepine-based agonists. The effect of *N*-methylation enhanced affinity (roughly 8-fold) and selectivity for 5-HT_{2c} receptors over 5-HT_{2a} and 5-HT_{2b}. Additional studies will be required to determine whether the positive effect of *N*-methylation can be extrapolated to other azepine-containing ligands, including lorcaserin. [^{11}C]**5** was reproducibly generated with good radiochemical yield, highlighting this approach for radiolabeling azepine-type ligands for more rapid screening of potential 5-HT_{2c} PET radiotracers.

dx.doi.org/10.1021/cn300223d | *ACS Chem. Neurosci.* 2013, 4, 261−265

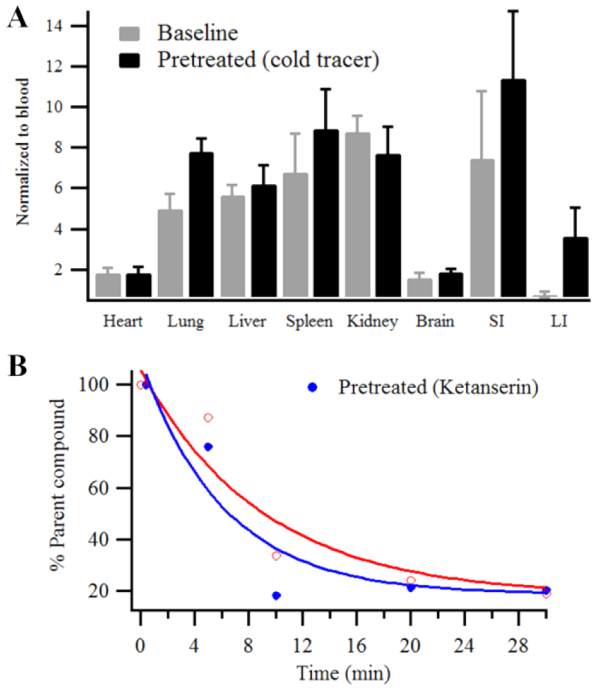


Figure 5. (A) Biodistribution of [^{11}C]**5** in rats at 90 min post injection (gray) and following pretreatment with unlabeled **5** (black). Data are normalized to blood. (SI = small intestine, LI = large intestine). (B) Plasma analysis for radioactive metabolites (data from baboon studies in Figure 4): baseline study (red); pretreated with ketanserin (blue).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experiment details, imaging protocol, and summary of image analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

This project was funded by a grant from the National Institute of Health (1R21MH093874).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The *in vitro* binding profile of several compounds was provided by the Psychoactive Drug Screening Program as part of the National Institute of Mental Health. The PDSP is overseen by Dr. Bryan Roth at the University of North Carolina Chapel Hill. The authors are grateful to Bryan McIntosh of Gamma Medica for assistance with the small animal PET scanner and wish to thank Grae Arabsz, Shirley Hsu, and Helen Deng for assistance during NHP imaging.

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