

Development of new brain imaging agents based upon nocaine–modafinil hybrid monoamine transporter inhibitors

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Abstract—¹¹C-labeled (+)-*trans*-2-[[[(3*R*,4*S*)-4-(4-chlorophenyl)-1-methylpiperidin-3-yl]methylsulfanyl]ethanol ([¹¹C]**5**) and (+)-*trans*-2-[[[(3*R*,4*S*)-4-(4-chlorophenyl)-1-methylpiperidin-3-yl]methylsulfanyl]-1-(piperidin-1-yl)ethanone ([¹¹C]**6**) were synthesized and evaluated as new imaging agents for the norepinephrine transporter (NET). [¹¹C]**5** and [¹¹C]**6** display high affinity for the NET in vitro (K_i = 0.94 and 0.68 nM, respectively) and significant selectivity over the dopamine (DAT) and serotonin transporters (SERT). Because of their high affinity and favorable transporter selectivities we speculated that these ligands might serve as useful PET agents for imaging NET in vivo. Contrary to our expectations, both of these ligands provided brain images that were more typical of those shown by agents binding to the DAT.

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The biogenic amine neurotransmitters, dopamine (DA), 5-hydroxytryptamine (5-HT), and norepinephrine (NE), are involved inter alia in the regulation of emotions, reactions to stress, and the physical drives of appetite and sleep.¹ Chemical imbalances in these neurotransmitters result in various pathological conditions such as depression, Parkinson's disease, bipolar disorder, and attention deficit hyperactivity disorder (ADHD).^{2–4} The reuptake of these monoamine transmitters into nerve terminals through neuronal plasma membrane monoamine transporters provides a mechanism for modulating the intensity and duration of monoamine signaling at synapses. Research with novel ligands that vary in their inhibitory potencies and selectivity profiles across the three brain monoamine transporters is of value for unraveling their associated pharmacology and for

the discovery of new medications (e.g., antidepressants) acting on these sites with fewer side-effects. In particular, positron emission tomography (PET) imaging probes for the serotonin transporter (SERT) and dopamine transporter (DAT) have proven useful in assessing transporter occupancy of established and novel medications in human subjects.^{5–7} Similar imaging studies targeting the norepinephrine transporter (NET) in human brain have lagged due to a lack of NET radioligands that give acceptable signals (high target to non-target radioactivity ratios). Recently, ¹¹C- and ¹⁸F-labeled analogs of reboxetine have been prepared and evaluated as in vivo markers of brain NET in rodent,⁸ monkey,^{9,10} and baboon.^{11,12} Such animal imaging studies have demonstrated selective binding of the *O*-[¹¹C]methyl and *O*-[¹⁸F]fluoromethyl analogs of reboxetine to the NET. Initial human imaging trials with the *O*-[¹¹C]methyl analog (named either [¹¹C]MeNER¹³ or [¹¹C]MRB¹⁴) have proceeded, although the signal from the binding of this radioligand to human NET is moderate. Moreover, a transient equilibrium for NET-specific binding of this analog is not reached during PET imaging and therefore

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quantitation of NET is challenging. There is thus a clear need for high affinity PET imaging agents that target NET and give high signals *in vivo*. Development of such PET radioligands, however, is particularly challenging owing to the relatively low density of NET sites⁸ in comparison to the other monoamine transporters (DAT and SERT). Thus, a possible complicating factor in the development of an effective NET radioligand is selectivity, with the putative NET radioligand competitively labeling these off-target transporters (*vide infra*).

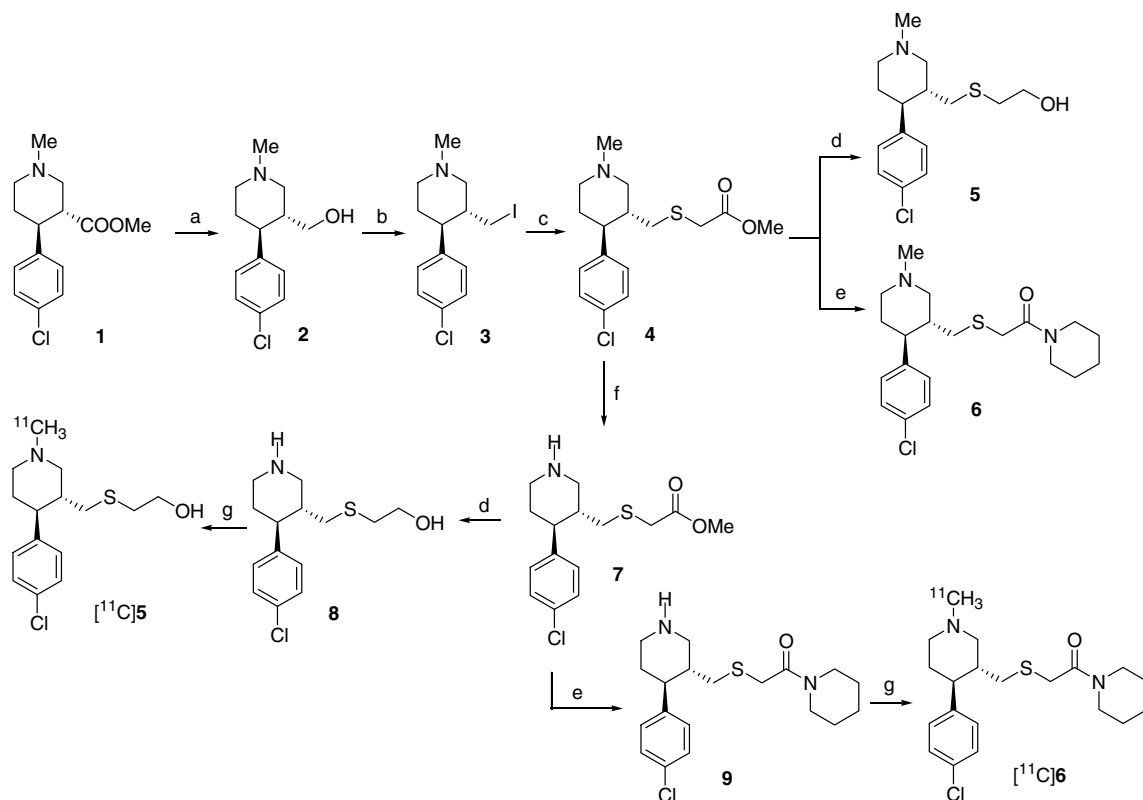
Recently, we described the synthesis of piperidine-based analogs of cocaine, one of which was found capable of antagonizing some of the effects of cocaine in animals, while showing a weaker reinforcing action in non-human primates in comparison to cocaine.¹⁵ In further research, this molecule, called nocaine, was modified by exploring the effect of replacing its ester group with a sulfur appendage like that found in the wake-promoting drug, modafinil.¹⁶ In these studies, we found that the alcohol **5** and amide **6** (Scheme 1) showed excellent affinity for the NET with K_i s of 0.94 ± 0.27 nM and 0.68 ± 0.25 nM (Table 1), respectively. The selectivity of the alcohol **5** for NET versus SERT is high as its K_i at the 5-HT transporter is 158 nM. Activity at the DAT was 16 nM, and thus the ligand displays a 17-fold higher inhibitory potency at the NET. Because of this high activity at the NET and moderate transporter selectivity, we were interested in exploring the capability of using the ligand for brain imaging with PET. Thus, we labeled these compounds with carbon-11 and evaluated

the ability of these novel radioligands to image the NET in rhesus monkey brain with PET.

Scheme 1 shows our synthesis of the target structures, starting from methyl 4β-(4-chlorophenyl)-1-methylpiperidine-3α-carboxylate **1**, according to the known literature procedure.¹⁶ One precursor for radiolabeling, **8**, was obtained by reduction of **7** with lithium aluminum hydride (LiAlH_4). Treatment of the ester **7** with piperidine afforded the other precursor for radiolabeling, namely **9**. Labeling of the target radioligands with carbon-11 proved straightforward and was carried out in a commercial module (Bioscan, Washington, DC) based on the HPLC loop radiomethylation method reported by Wilson et al.¹⁸ Unoptimized and non-decay-corrected isolated radiochemical yields (based on starting $[^{11}\text{C}]\text{CO}_2$) for $[^{11}\text{C}]\mathbf{5}$ and $[^{11}\text{C}]\mathbf{6}$ ranged from 12% to 14% with specific radioactivities of >93 GBq/ μmol (2.5 Ci/ μmol) at end-of-synthesis.

PET imaging experiments with $[^{11}\text{C}]\mathbf{5}$ or $[^{11}\text{C}]\mathbf{6}$ were conducted in rhesus monkeys using a GE Advance camera (FWHM = 7.0 mm) or HRRT (FWHM = 3.0 mm; Siemens/CPS, Knoxville, TN, USA). For each radioligand, a two-scan protocol, comprising first a baseline and then a desipramine pre-blocking experiment, was performed on the same day. The resulting decay-corrected time-radioactivity curves are shown in Chart 1 (A and B).

In baseline scans $[^{11}\text{C}]\mathbf{5}$ and $[^{11}\text{C}]\mathbf{6}$ showed acceptable brain penetration with maximal whole brain uptake of



Scheme 1. Reagents and conditions: (a) LiAlH_4 , THF, 92%; (b) $\text{Ph}_3\text{P/I}_2/\text{imidazole}$, 86%; (c) methyl thioglycolate, Cs_2CO_3 , 88%; (d) LiAlH_4 , THF, 56%; (e) piperidine, MeOH; (f) 1-chloroethyl chloroformate, proton sponge, CH_2Cl_2 , 80%; (g) $[^{11}\text{C}]\text{I}$, DMF.

Table 1. In vitro inhibition at monoamine transporters for candidate PET ligands ($K_i \pm \text{SEM}$ (nM))

Compound	<i>c</i> Log <i>P</i>	Reuptake K_i (nM)			Selectivity (based on K_i)		
		[³ H]DA	[³ H]5-HT	[³ H]NE	5-HT/DA	NE/DA	NE/5-HT
Cocaine ^a		423 ± 147	155 ± 1	108 ± 4	0.37	0.26	0.7
Desipramine ^b		78720	61	4	0.0007	0.00005	0.066
5 ^a	2.81	16.1 ± 4.5	158.0 ± 4.6	0.94 ± 0.27	9.81	0.06	0.005
6 ^a	3.80	83.3 ± 1.2	4.5 ± 0.7	0.68 ± 0.25	0.05	0.008	0.15

^a Data for cocaine, **5**, and **6** were taken from Ref. 16.^b Data for desipramine were taken from Ref. 17.

0.045% ID/g at 20 min and 0.021% ID/g at 9 min, respectively. For both radioligands the magnitudes of regional brain radioactivity concentrations were in the

order: striatum > thalamus > cerebellum. Striatal uptake of [¹¹C]**5**, for example, was clearly evident in PET images 30 min after radiotracer injection (Fig. 1).

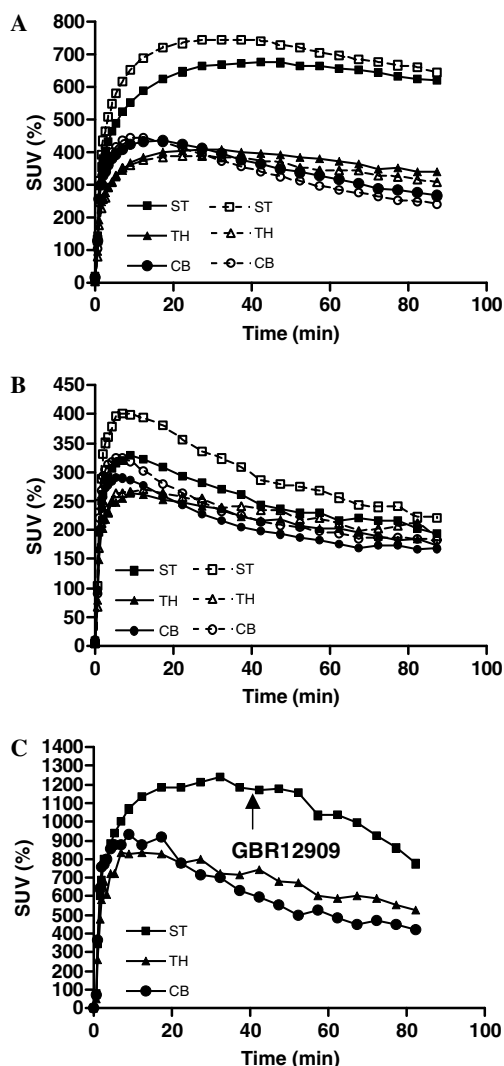


Chart 1. Decay-corrected time-radioactivity curves of [¹¹C]**5** (A) and [¹¹C]**6** (B) in regions of rhesus monkey brain. Solid lines and symbols are from a baseline scan. Dashed lines and open symbols are from a second scan where the animal was treated with the NET blocker, desipramine (5 mg/kg, iv), at 20 min before radioligand injection. Time-radioactivity curve of [¹¹C]**5** where the DAT blocker GBR 12909 di-HCl (5 mg/kg, iv) was administered at 40 min after radioligand injection is shown in (C). Key, ST: striatum; TH: thalamus; CB: cerebellum. Radioligand doses were 167–185 MBq (4.5–5 mCi) with specific radioactivities of >40.7 GBq/μmol (1.1 Ci/μmol) at the time of injection. SUV (%) = %ID/g × gram animal body weight.

This regional distribution is inconsistent with that known for NET where thalamic density is higher than striatal density.^{12,19} Pretreatment of monkeys with the NET inhibitor, desipramine, followed by injection of either [¹¹C]**5** or [¹¹C]**6** resulted in minimal reduction of radioactivity in the NET-rich thalamus. The 5 mg/kg intravenous dose of desipramine used in our experiments has previously been shown to be effective in blocking the uptake of other NET radioligands in monkey.¹⁰ Thus, despite their subnanomolar affinities in vitro for NET, [¹¹C]**5** and [¹¹C]**6** appear poorly suited for in vivo labeling of NET sites in monkey brain.

Owing to the high striatal uptake for [¹¹C]**5** observed in the baseline monkey scan²⁰, it was hypothesized that this *N*-methyl piperidine exhibited specific binding to DAT. To test this hypothesis, a PET experiment was performed in rhesus monkey wherein, at 40 min after injection of [¹¹C]**5**, a dose of the DAT ligand, GBR 12909, was administered (Chart 1C). Inspection of the striatal time-radioactivity curve revealed a markedly decreasing radioactivity content after injection of GBR 12909, suggesting that [¹¹C]**5** shows specific binding to

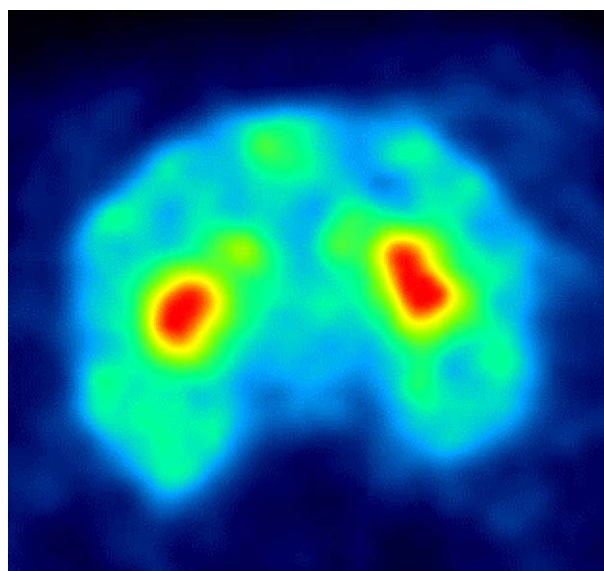


Figure 1. Transaxial PET image of Rhesus monkey brain 30 min after injection of [¹¹C]**5** demonstrating pronounced uptake of radiotracer in the DAT-rich striatum.

DAT. Cocaine, which possesses a 25-fold lower affinity for DAT than **5** ($K_i = 423 \text{ nM}$ ¹⁶), has been labeled with carbon-11 and shown to image the highly abundant brain DAT in vivo.²¹ Hence, in retrospect, it is not too unexpected that [¹¹C]**5** (DAT $K_i = 16 \text{ nM}$) also labels and images DAT in vivo.

In conclusion, PET data given for the two ligands presented in this paper allowed us to state that the first generation of ¹¹C-labeled *N*-methyl piperidines shows an ability to label DAT. While further investigation will be needed to demonstrate whether these particular ligands possess any advantage over the DAT agents currently used in tomographic imaging,²² it is clear that ligands of much higher NET affinity and selectivity will be required from this structural class in order to identify in vivo imaging tools for the NET. Further extensions of the SAR studies on this type of ligands are likely to afford the opportunity to discover new NET inhibitors with higher potency and selectivity that are suitable for the PET imaging. Related work is underway and will be reported in due course.

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