



Published in final edited form as:

ACS Chem Neurosci. 2015 December 16; 6(12): 1965–1971. doi:10.1021/acschemneuro.5b00223.

In Vivo Metabolic Trapping Radiotracers for Imaging Monoamine Oxidase-A and –B Enzymatic Activity

Allen F. Brooks[†], Xia Shao[†], Carole A. Quesada[†], Phillip Sherman[†], Peter J.H. Scott^{*,†,‡}, and Michael R. Kilbourn^{*,†}

[†]Division of Nuclear Medicine, Department of Radiology, University of Michigan Medical School, Ann Arbor, Michigan 48109

[‡]The Interdepartmental Program in Medicinal Chemistry, University of Michigan, Ann Arbor, Michigan 48109

Abstract

The isozymes of monoamine oxidase (MAO-A and MAO-B) are important enzymes involved in the metabolism of numerous biogenic amines, including the neurotransmitters serotonin, dopamine and norepinephrine. Recently, changes in concentrations of MAO-B have been proposed as an *in vivo* marker of neuroinflammation associated with Alzheimer's disease. Previous developments of *in vivo* radiotracers for imaging changes in MAO enzyme expression or activity have utilized the irreversible propargylamine-based suicide inhibitors, or high-affinity reversibly-binding inhibitors. As an alternative approach, we have investigated 1-[¹¹C]methyl-4-aryloxy-1,2,3,6-tetrahydropyridines as metabolic trapping agents for the monoamine oxidases. MAO-mediated oxidation and spontaneous hydrolysis yields 1-[¹¹C]methyl-2,3-dihydro-4-pyridinone as a hydrophilic metabolite that is trapped within brain tissues. Radiotracers with phenyl, biphenyl and 7-coumarinyl ethers were evaluated using microPET imaging in rat and primate brain. No isozyme selectivity for radiotracer trapping was observed in the rat brain for any compound, but in the monkey brain the phenyl ether demonstrated MAO-A selectivity, and the coumarinyl ether showed MAO-B selectivity. These are lead compounds for further development of 1-[¹¹C]methyl-4-aryloxy-1,2,3,6-tetrahydropyridines with optimized brain pharmacokinetics and isozyme selectivity.

Keywords

Alzheimer's disease; neuroimaging; positron emission tomography; carbon-11; monoamine oxidase

The enzymes monoamine oxidase-A (MAO-A) and –B (MAO-B) are responsible for the oxidation of a wide variety of amines, including the amine neurotransmitters dopamine,

*Corresponding Author: Michael R. Kilbourn. mkilbour@umich.edu. Tel.: +1 (734) 763-9246.; Peter J. H. Scott. pjhscott@umich.edu. Tel.: +1(734)615-1756.

Spectra for all novel compounds synthesized, chromatographic data for radiotracer purifications, and procedures for microPET imaging. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Author Contributions

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

norepinephrine, and serotonin. Inhibitors of monoamine oxidases (MAOIs) are important in clinical medicine: inhibition of MAO-A is utilized in the management of depression, and inhibition of MAO-B forms one of the therapeutic approaches to treating Parkinson's disease¹. More recently, the potential for using changes of MAO-B as a marker of astrogliosis in neurodegenerative diseases (such as Alzheimer's disease) has become of interest². The importance of the monoamine oxidases in numerous disease processes stimulated interest for *in vivo* non-invasive imaging of changes of MAO in the human brain, leading to the development of numerous carbon-11 and fluorine-18 labeled radiotracers useful for Positron Emission Tomography (PET) studies³. Prior approaches to isoform-selective MAO radiotracer development have mostly concentrated on (a) irreversibly trapped suicide inhibitors (e.g., [¹¹C]deprenyl and [¹¹C]clorgyline) that form covalent bonds between the radiotracer and the flavin cofactor of the enzymes, and (b) reversibly-binding high-affinity inhibitors (e.g., [¹¹C]befloxatone, [¹¹C]harmine). Several of these have been successfully introduced into human PET studies, including evaluation in Alzheimer's disease⁴, but there is continued interest in the development of new MAO radiotracers labeled with carbon-11 and fluorine-18 in the search for optimal *in vivo* radiotracers^{3b, 5}.

An alternative approach for measuring enzymatic activity *in vivo* is the use of metabolic trapping, where the product of the catalytic activity of the enzyme is not covalently bound but still retained within the target tissue. This is the mechanism behind such *in vivo* imaging radiotracers as 2-[¹⁸F]fluoro-2-deoxyglucose ([¹⁸F]FDG, glucose metabolism), N-[¹¹C]methyl-4-piperidinypropionate or acetate ([¹¹C]PMP and [¹¹C]MP4A, acetylcholinesterase)⁶, and [¹⁸F]fluoroDOPA (dopamine synthesis) among other examples. The concept of non-covalent metabolic trapping has been applied to MAO but the effort has been severely limited. The syntheses of radiolabeled forms of N,N-dimethylphenylethylamine were reported, initial biodistribution studies reported in rodents, and even a single human PET image presented⁷. Preliminary studies in both rat and primate were also done with carbon-11 labeled MPTP⁸ and a radioiodinated tetrahydropyridine (N-methyl-4-(4'-hydroxy-3'-[¹²⁵I]iodophenyl)-1,2,3,6-tetrahydropyridine)⁹, but no additional reports were made for use of either compound. Further development and validation (demonstration of isoform selectivity, pharmacokinetic analyses) of non-covalent metabolic trapping radiotracers for MAO have not been pursued, and there have been no applications of this concept to studies of MAO in human diseases.

We have investigated here the metabolic trapping of 1-methyl-4-aryloxy-1,2,3,6-tetrahydropyridines, molecules that undergo an initial single oxidation by monoamine oxidases (Fig. 1), forming dihydropyridinium species that are then rapidly hydrolyzed to a phenol and 1-methyl-2,3-dihydro-4-pyridinone¹⁰. As that ketone is hydrophilic (cLog P = -0.18), labeling with a carbon-11 at the N-methyl substituent was hypothesized to provide a metabolite that would be trapped within brain tissues. Furthermore, as a second oxidation step of the dihydropyridinium intermediate never occurs, the 4-aryloxy-1,2,3,6-tetrahydropyridines are non-toxic (in contrast to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)). A series of 4-aryloxy substituted tetrahydropyridines were synthesized by Castagnoli and coworkers¹¹ and evaluated as substrates for the monoamine oxidases; those studies demonstrated that a broad range of *in vitro* kinetic parameters (K_m and K_{cat}) and MAO isoform selectivity are possible by selection of the 4-aryloxy substituent.

RESULTS AND DISCUSSION

Chemistry

Using the prior studies of the *in vitro* kinetics of MAO-mediated hydrolysis as a guide, three target compounds were selected to test the concept of using 1-methyl-4-aryloxy-1,2,3,6-tetrahydropyridines as isoform-selective MAO-catalyzed metabolic radiotracers (Fig. 2). The simplest molecule, 1-methyl-4-phenyloxy-1,2,3,6-tetrahydropyridine (PHXY, **1a**), was reported as a mixed MAO-A/MAO-B substrate with a slight (2-fold) higher reactivity towards MAO-B¹². The biphenyl-substituted derivative (BiPHEN, **1c**) demonstrated 8-fold higher *in vitro* reactivity towards MAO-A over MAO-B (Wang and Castagnoli 1995)^{11a}. Finally, the 7-coumarinyl derivative (COU, **1b**) was reported by Long and coworkers¹³ to have a 22-fold selectivity for MAO-B.

The syntheses of 1-methyl-4-aryloxy-1,2,3,6-tetrahydropyridines in isotopically unmodified forms were done following modifications of the literature procedure^{11a, 13} and utilized the reaction of 4-chloro-1-methylpyridin-1-ium triflate with the appropriate phenols (**2a–c**) to form intermediate 1-methyl-4-aryloxy-pyridines (**3a–c**), followed by sodium borohydride reduction of the pyridine ring (Scheme 1). The products **1a–c** were isolated in good yields (12–47%) using flash silica gel chromatography. The syntheses of the carbon-11 forms of PHXY, COU and BiPHEN were done using a one-pot procedure of N-[¹¹C]methylation of the 4-aryloxy-pyridines **4a–c** using no-carrier-added [¹¹C]methyl triflate, followed by rapid (5 min) sodium borohydride reduction in ethanolic solution (Scheme 1). The required pyridine precursors (**4a–c**) were prepared by reaction of 4-chloropyridinium chloride with the corresponding phenols **2a–c**. The radiotracers ([¹¹C]PHXY (**1a**), [¹¹C]COU (**1b**) and [¹¹C]BiPHEN (**1c**)) were isolated and purified by HPLC, with overall synthesis times of 30 min. Although isolated radiochemical yields were low (1–5%, not corrected for decay) they were not optimized, and specific activities averaged >55.5 TBq/mmol. Radiotracers were then formulated in isotonic saline for microPET imaging studies in rats and monkey.

Biology

The *in vivo* brain distributions of all three radiotracers were evaluated using microPET imaging, with the initial evaluations of pharmacokinetics and isozyme selectivity performed in the rat brain. However, due to the well-known potential for species differences in the selectivity and rates of substrate oxidations by the monoamine oxidases^{7a, 14}, additional studies were performed in the rhesus monkey.

The microPET studies in rat brain demonstrated similar pharmacokinetics for [¹¹C]PHXY and [¹¹C]COU, with rapid initial uptakes peaking at 90–150 seconds and equivalent maximum brain concentrations (1.1 % injected dose/g for both). For both [¹¹C]PHXY and [¹¹C]COU, there followed a rapid washout of radioactivity until constant levels of trapped radioactivity were reached by 20–30 min, with trapping fractions (plateau/peak) of 55–60% for [¹¹C]PHXY and 10–15% for [¹¹C]COU. [¹¹C]BiPHEN showed different pharmacokinetics, with a significantly lower and broader initial uptake (0.43 % injected dose/g) and a more gradual washout that did not quite reach a constant level by the end of the 60 min imaging period.

To test for isozyme selectivity, studies of trapping efficiencies were performed after pretreatments with deprenyl (selective MAO-B inhibitor, 10 mg/kg i.p. 90 min prior to scan) or clorgyline (selective MAO-A inhibitor, 10 mg/kg i.p. 90 min prior to scan) or both irreversible inhibitors. The *in vivo* trapping of [¹¹C]PHXY and [¹¹C]BiPHEN were more sensitive to MAO-A inhibition, and [¹¹C]COU more sensitive to MAO-B inhibition, but none of the three radiotracers examined here exhibited specificity for either isozyme in the rat brain. The pharmacological blocking studies support little non-specific binding of [¹¹C]PHXY and [¹¹C]COU, as residual trapped radioactivity levels are very low after enzyme inhibition. The lower brain uptake and slower kinetics of [¹¹C]BiPHEN suggests the radiotracer might exhibit higher non-specific distribution, consistent with its higher lipophilicity.

The rat studies were encouraging and supported the hypothesis that MAO-mediated oxidation of 1-methyl-4-aryloxy-1,2,3,6-tetrahydropyridines could be imaged in the mammalian brain. Metabolite studies of rat brain extracts at 10 minutes after injection of [¹¹C]PHXY confirmed the formation of a single polar radioactive metabolite, consistent with formation of 1-[¹¹C]methyl-2,3-dihydro-4-pyridinone. The failure to achieve isozyme selectivity (or specificity) for any of the radiotracers in the rat brain was however not discouraging. The prior studies^{11a, 12} of 1-methyl-4-aryloxy-1,2,3,6-tetrahydropyridines had utilized MAO isolated from bovine or human tissues (or both), and numerous studies have demonstrated significant species variability in the behavior of both inhibitors and substrates towards the two isozymes of MAO^{7a, 14}. This variability supported the notion that our new radiotracers needed to be evaluated in a second species.

The imaging studies in the rhesus monkey brain showed pharmacokinetics of all three radiotracers that were different than seen in the rat brain, with more encouraging isozyme selectivity for [¹¹C]PHXY and [¹¹C]COU. [¹¹C]PHXY, the lowest molecular weight and least lipophilic compound, exhibited the highest uptake and trapping, with clear heterogeneity between the major regions of the brain (striatum > thalamus > cerebellum > cortex) (Fig. 4A). The coumarin derivative [¹¹C]COU showed similar pharmacokinetics, with slightly lower trapping of radioactivity, and little or no distinction between levels in the striatum and thalamus (Fig 4D). Finally, the kinetics of [¹¹C]BiPHEN were significantly different, with much lower brain uptake and trapping, and less heterogeneity in the regional brain distribution (Fig. 4G). No attempts have been made to correlate the observed regional radiotracer trapping concentrations with *in vitro* values for protein concentrations or enzyme activities of MAO-A or MAO-B, as there is very limited data available for rhesus monkey brain¹⁵. The rank order of striatum > cerebellar cortex > cortex for [¹¹C]COU is however consistent with the *in vitro* studies of Riachi and Harik^{15a} using either binding of [³H]pargyline or oxidation of substrates (MPTP, benzylamine).

Pharmacological studies for isozyme selectivity in the monkey were done using the reversible MAO inhibitors moclobemide (MAO-A, 1.0 mg/kg i.v.) and lazabemide (MAO-B, 0.5 mg/kg i.v.), to avoid the long-lasting irreversible inactivation of the enzymes known to occur with deprenyl and clorgyline¹⁶. The doses were administered as a 10 min infusion prior to radiotracer injection. The doses of inhibitors were limited to amounts supported by prior safe administration to monkeys, based on literature reports; without independent

evidence that the doses were sufficient for complete inhibition of enzyme activities, or that the radiotracers themselves do not effectively displace these reversibly-bound inhibitors. These studies with moclobemide and lazabemide are considered here as pharmacological intervention studies but not necessarily full blocking studies (in the sense the enzymes can be fully and irreversibly inhibited, as was achieved with deprenyl and cloglyline in the rat brain).

Radiotracer trapping of the simplest substrate [^{11}C]PHXY was significantly reduced in all brain regions by inhibition of MAO-A by moclobemide, with data for striatum and cortex shown in Figs 4B–C. In contrast, administration of the MAO-B inhibitor lazabemide produced no change in the cortex and a small and perhaps insignificant increase in the striatum. These preliminary results suggest that, contrary to the *in vitro* assays which used a combination of enzymes from human (MAO-A) and bovine (MAO-B) tissue sources and contrary to the rat imaging studies, [^{11}C]PHXY exhibits MAO-A selectivity in the monkey brain.

As was hoped based on the reported *in vitro* selectivity of [^{11}C]COU, where human tissues provided the sources for both MAO-A and MAO-B, the *in vivo* blocking studies in the monkey brain showed a significant reduction in trapping in striatum and cortex after inhibition of MAO-B (Fig. 4E–F), with lesser effects for inhibition of MAO-A. Again, the monkey imaging studies are more encouraging than those of the rat where significant effect of both MAO-A and MAO-B inhibition were observed for [^{11}C]COU. These studies emphasize the importance of species selection when evaluating MAO substrates or inhibitors.

Finally, the results for [^{11}C]BiPHEN, a compound which was more selective for MAO-A *in vitro* (using human and bovine sources of MAO), were less clear. Inhibition of MAO-B had mixed effects on [^{11}C]BiPHEN pharmacokinetics, and the inhibition with the MAO-A inhibitor moclobemide produced an unexpected increase in trapping of radioactivity in all brain regions (Figs. 4 H–I). One possible explanation is that moclobemide inhibition of peripheral MAO-A activity increased the available [^{11}C]BiPHEN in the blood, resulting in increased radiotracer delivery to the brain. We had observed such changes in brain pharmacokinetics in previous studies of radiolabeled trapping agents for the enzyme acetylcholinesterase, where inhibition of blood enzymes significantly increased radiotracer delivery to the brain. Studies of [^{11}C]BiPHEN employing metabolite-corrected blood input data would allow us to verify this hypothesis, however, the lower initial brain uptake, poor regional heterogeneity, mixed pharmacological selectivity and potential for significant non-specific distribution due to its high lipophilicity ($\text{clog } P = 4.72$) make [^{11}C]BiPHEN at this point a less interesting candidate MAO-A radiotracer than [^{11}C]PHXY.

SUMMARY

These preliminary studies have demonstrated the feasibility of using 1-methyl-4-aryloxy-1,2,3,6-tetrahydropyridines as substrates for *in vivo* PET imaging studies of the enzymatic activity of brain monoamine oxidases. Two of the initial radiotracers examined here, [^{11}C]PHXY and [^{11}C]COU, already show encouraging isozyme selectivity in the

monkey brain, and are of immediate interest for further evaluation. The general synthetic strategy shown in Scheme I can be used to prepare a wide variety of substituted tetrahydropyridines in the continued search for radiotracers having the optimal combinations of lipophilicity, affinity for the enzyme (K_m) and rate of enzymatic hydrolysis (K_{cat}). Additional modifications of the rate of MAO-mediated oxidation of 1-methyl-4-aryloxy-1,2,3,6-tetrahydropyridines should also be possible by selective incorporation of deuterium at the 6-position of the tetrahydropyridine ring, as demonstrated to be effective for MPTP¹⁷.

These studies also demonstrate some of the difficulties in developing new radiotracers for *in vivo* imaging studies of the monoamine oxidases. The potential for significant species differences is well recognized for the isozymes of MAO, and were reflected in the relatively little selectivity of [¹¹C]PHXY and [¹¹C]COU in the rat brain. A correlation of regional *in vivo* brain trapping of our new radiotracers with *in vitro* values for enzyme activities is essentially impossible given the extreme paucity of available data on the regional distributions of enzymatic activities in the monkey brain. Finally, pharmacological studies in the monkey have to be planned carefully, as the use of irreversible inhibitors (e.g., deprenyl and cloglyline) that unequivocally block enzyme action poses a challenge given the now recognized long-lasting effects of such inhibitors on the concentrations of active enzyme molecules¹⁶.

EXPERIMENTAL SECTION

Chemistry

General Considerations—All solvents and reagents were commercially available and used without further purification unless otherwise stated. 7-Methylumbeliferone and 3-phenylphenol were obtained from Sigma-Aldrich; 4-Phenoxypyridine was purchased from TCI America and used directly as a precursor for radiolabeling. NMR spectra were recorded with a Varian 400 MHz instrument at room temperature with tetramethylsilane (TMS) as an internal standard. Mass spectra were performed on a Micromass LCT Time-of-Flight mass spectrometer or an Agilent Q-TOF HPLC-MS employing the electrospray ionization (ESI) method. High performance liquid chromatography (HPLC) was performed using a Shimadzu LC-2010A HT system equipped with a Bioscan B-FC-1000 radiation detector.

General Procedure for preparation of 1-methyl-4-aryloxy-1,2,3,4-tetrahydropyridines (1a–c)—Phenol starting material (0.72 mmol) was added to sodium methoxide (0.87 mmol) dissolved in DMF (3 mL) and stirred for 10 minutes. 4-Chloro-1-methylpyridin-1-ium triflate (0.73 mmol) was added and the reaction was stirred for 18 h. The solvent was removed in vacuo and the resulting intermediate was suspended in methanol (3 mL). The reaction mixture was cooled to 0 °C in a water ice bath, and NaBH₄ (2.9 mmol) was added slowly. After 1 h the solvent was removed in vacuo. Water and ethyl acetate were added to the residue and the mixture was transfer to a separatory funnel. The product was extracted with ethyl acetate (3X), dried over sodium sulfate, filtered, and concentrated in vacuo. The product was purified by flash silica gel chromatography (dichloromethane, methanol gradient).

1-Methyl-4-phenoxy-1,2,3,6-tetrahydropyridine (PHXY, 1a)—Starting from 4-phenoxy-pyridine, the reduction step reaction yielded 0.056 g (41 % yield) of **1a** as a white solid. ¹H NMR (400 MHz; CH₃OD-d₄)/δ (ppm): 7.32 (2H, t, J= 7.8, 2H), 7.08 (1H, t, J=7.4), 7.00 (2H, d, J= 7.8), 4.77 (1H, t, J= 3.2), 2.98 (2H, d J=3.1), 2.70 (2H, t, J=5.9), 2.38 (2H, m), 2.37 (3H, s); HRMS: calculated for [M+H]⁺(M = C₁₂H₁₅NO), 190.1226, found 190.1230.

4-Methyl-7-((1-methyl-1,2,3,6-tetrahydropyridin-4-yl)oxy)-2H-chromen-2-one (COU, 1b)—Starting with 4-methylumbeliferone, the reaction sequence yielded 0.091 g (47 % yield) of **1b** as an off-white solid. ¹H NMR (400 MHz; CH₃OD-d₄)/δ (ppm): 7.74 (1H, d, J=8.7), 7.06 (1H, dd, J=2.4, 8.7), 7.00(1H, d, J=2.4), 6.23(1H, d, J=1.1), 5.21 (1H, t, J=3.4); 3.17 (2H, d, J=3.4) 2.84 (2H, t, J=5.9), 2.48 (3H, s), 2.46 (3H, s), 2.42 (2H, m); HRMS: calculated for [M+H]⁺(M = C₁₆H₁₇NO₃), 272.1281, found 272.184.

4-([1,1'-Biphenyl]-3-yloxy)-1-methyl-1,2,3,6-tetrahydropyridine (BiPHEN, 1c)—Starting from 3-hydroxybiphenyl, the reaction sequence yielded 0.0743 g (39 % yield) of **1c** as a pale yellow oil. ¹H NMR (400 MHz; CH₃OD-d₄)/δ (ppm): 7.57 (2H, d, J=7.2), 7.42-7.30 (5H, m), 7.27 (1H, t, J=1.9), 6.98 (1H, dq J=7.8, 1.2), 4.86 (1H, t, J=3.5), 2.96 (2H, dd, J=5.8, 2.5), 2.67 (2H, t, J=5.9) 2.39 (2H, m), 2.34 (3H, s); HRMS: calculated for [M+H]⁺(M = C₁₈H₁₉NO), 266.1539, found 266.1538.

General Procedure for preparation of 4-aryloxy-pyridines (4b,c)—Phenol starting material (1.6 mmol) was added to potassium *tert*-butoxide (2.9 mmol) dissolved in DMF (7 mL). The reaction mixture was heated to 140 °C, 4-chloropyridin-1-ium (1.33 mmol) was added, and the reaction was stirred for 18 h. The reaction was cooled to room temperature and quenched with aqueous saturated NH₄Cl. The product was extracted with ethyl acetate (3X), dried over sodium sulfate, filtered, and concentrated in vacuo. The product was purified by flash silica gel chromatography (hexanes, ethyl acetate gradient).

4-Methyl-7-(pyridin-4-yloxy)-2H-chromen-2-one (4b)—Starting with 4-methylumbeliferone the reaction yielded 0.040 g (12 % yield) of **4b** as an off-white solid. ¹H NMR (400 MHz; CH₃OD-d₄)/δ (ppm): 8.48 (2H, d, J=6.1), 7.88 (1H, d, J=9.3), 7.170-7.155 (2H, m), 7.08 (2H, d, J=6.1), 6.33 (1H, s), 2.50 (3H, s); HRMS: calculated for [M+H]⁺(M = C₁₅H₁₁NO₃), 254.0812, found 254.0815.

4-([1,1'-Biphenyl]-3-yloxy)pyridine (4c)—Starting with 3-hydroxybiphenyl the reaction yielded 0.1831 g (56 % yield) of **4c** as a yellow solid. ¹H NMR (400 MHz; CH₃OD-d₄)/δ (ppm): 8.41 (2H, d, J=4.9, 1.5), 7.62 (2H, d, J=7.5), 7.55 (2H, m), 7.44 (2H, t, J=7.5), 7.40-7.34 (2H, m), 7.13 (1H, dt, J=7.1, 2.2), 7.00 (2H, dd, J=4.9, 1.5); HRMS: calculated for [M+H]⁺(M = C₁₇H₁₃NO), 248.1070, found 248.1068.

Radiochemistry

General Considerations—Reagents and solvents were commercially available and used without further purification, unless otherwise noted: sodium chloride (0.9% USP) and sterile water for Injection (USP) were purchased from Hospira; Dehydrated Alcohol for Injection

(USP) was obtained from Akorn Inc. Shimalite-Nickle was purchased from Shimadzu; iodine was obtained from EMD; phosphorus pentoxide was acquired from Fluka; molecular sieves were purchased from Alltech; and HPLC columns were acquired from Phenomenex. Other synthesis components were obtained as follows: sterile filters were acquired from Millipore; C18-light Sep-Paks and Porapak Q were purchased from Waters Corporation; 10 cc sterile vials were obtained from HollisterStier. Sep-Paks were flushed with 10 mL of ethanol followed by 10 mL of sterile water prior to use.

General Procedure for Radiochemical Syntheses—Production of carbon-11 labeled radiotracers was carried out using a TracerLab FX_{C-Pro} automated radiochemistry synthesis module (General Electric, GE). [¹¹C]Carbon dioxide was produced using a GE PETTrace cyclotron (40 μA beam for 20 min) and converted by standard procedures into carbon-11 labeled methyl triflate ([¹¹C]CH₃OTf). The [¹¹C]CH₃OTf in helium carrier gas was bubbled into a vial containing a solution of precursor (1 mg) dissolved in ethanol (0.2 mL). At completion of transfer of radioactivity into the reaction vial, the ethanol solution was then transferred to a second conical vial containing sodium borohydride (2 mg) in ethanol (0.3 mL). The resulting mixture was stirred for 5 min at room temperature and then the reaction was quenched by addition of HPLC buffer. The crude product was loaded onto a semi-preparative HPLC loop. The product was purified by reverse phase chromatography (Prodigy ODS prep, 250 × 10 mm, 10μ, 4 mL/min), collected and diluted into H₂O (40 mL) and reformulated using a C-18 extraction disk into a final 5 mL total volume of 10 % ethanol in saline. The doses produced were assessed via standard quality control techniques and were appropriate for rodent and non-human primate studies. Average specific activity was 69116 GBq/mmol (range of 23236-178081 GBq/mmol). Overall synthesis times were 30 min from end-of-bombardment.

[¹¹C]1-Methyl-4-phenoxy-1,2,3,6-tetrahydropyridine ([¹¹C]PHXY, [¹¹C]1a)—In non-optimized yields, 270 ± 173 mBq of the phenyl ether (0.8 % yield from [¹¹C]CH₃OTf, not decay corrected; n=8) were collected. The product was purified by semi-preparative reverse phase chromatography (250 × 10, 10μ, ODS prep column eluted with 40 % CH₃CN, 60 % H₂O, 10 mM NH₄OAc).

[¹¹C]4-Methyl-7-(pyridin-4-yloxy)-2H-chromen-2-one ([¹¹C]COU, [¹¹C]1b)—In non-optimized yields, 577 ± 141 MBq of the coumarin ether (1.7 % yield from [¹¹C]CH₃OTf, not decay corrected; n=10) were collected. The product was purified by semi-preparative reverse phase chromatography (250 × 10, 10μ, ODS prep column eluted with 30 % CH₃CN, 70 % H₂O, 10 mM NH₄OAc).

[¹¹C]4-([1,1'-Biphenyl]-3-yloxy)-1-methyl-1,2,3,6-tetrahydropyridine ([¹¹C]BiPHEN, [¹¹C]1c)—In non-optimized yields, 551 ± 126 MBq of the biphenyl ether (1.7 % yield from [¹¹C]CH₃OTf, non-decay corrected; n=7). The product was purified by semi-preparative reverse phase chromatography (250 × 10, 10μ, ODS prep column eluted with 50 % CH₃CN, 50% H₂O, 10 mM NH₄OAc, pH 4.5).

Acknowledgments

Funding

Financial support for this work from the National Institutes of Health (National Institute of Neurological Diseases and Stroke, Award Number 1R21 NS075553 (MRK), and National Institute of Biomedical Imaging and Bioengineering, Award Number T32-EB005172 (PJHS, MRK) is gratefully acknowledged. The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Additional funding for this research from the Alzheimer's Association (NIRP-14-305669 (PJHS)) is also acknowledged.

References

1. Finberg JPM. Update on the pharmacology of selective inhibitors of MAO-A and MAO-B: Focus on modulation of CNS monoamine neurotransmitter release. *Pharmacology & Therapeutics*. 2014; 143(2):133–152. [PubMed: 24607445]
2. Jacobs AH, Tavitian B. Noninvasive molecular imaging of neuroinflammation. *J Cereb Blood Flow Metab*. 2012; 32(7):1393–1415. [PubMed: 22549622]
3. (a) Kersemans K, Van Laeken N, De Vos F. Radiochemistry devoted to the production of monoamine oxidase (MAO-A and MAO-B) ligands for brain imaging with positron emission tomography. *Journal of Labelled Compounds and Radiopharmaceuticals*. 2013; 56(3–4):78–88. [PubMed: 24285313] (b) Fowler JS, Logan J, Shumay E, Alia-Klein N, Wang GJ, Volkow ND. Monoamine oxidase: radiotracer chemistry and human studies. *Journal of Labelled Compounds and Radiopharmaceuticals*. 2015; 58(3):51–64. [PubMed: 25678277]
4. (a) Carter SF, Schöll M, Almkvist O, Wall A, Engler H, Långström B, Nordberg A. Evidence for Astrocytosis in Prodromal Alzheimer Disease Provided by 11C-Deuterium-L-Deprenyl: A Multitracer PET Paradigm Combining 11C-Pittsburgh Compound B and 18F-FDG. *Journal of Nuclear Medicine*. 2012; 53(1):37–46. [PubMed: 22213821] (b) Choo I, Carter S, Schöll M, Nordberg A. Astrocytosis measured by 11C-deprenyl PET correlates with decrease in gray matter density in the parahippocampus of prodromal Alzheimer's patients. *Eur J Nucl Med Mol Imaging*. 2014; 41(11):2120–2126. [PubMed: 25077930]
5. Tong J, Meyer JH, Furukawa Y, Boileau I, Chang LJ, Wilson AA, Houle S, Kish SJ. Distribution of monoamine oxidase proteins in human brain: implications for brain imaging studies. *J Cereb Blood Flow Metab*. 2013; 33(6):863–871. [PubMed: 23403377]
6. Kikuchi T, Okamura T, Zhang MR, Irie T. PET probes for imaging brain acetylcholinesterase. *Journal of Labelled Compounds and Radiopharmaceuticals*. 2013; 56(3–4):172–179. [PubMed: 24285323]
7. (a) Inoue H, Castagnoli K, Van der Schyf C, Mabic S, Igarashi K, Castagnoli N. Species-Dependent Differences in Monoamine Oxidase A and B-Catalyzed Oxidation of Various C4 Substituted 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridinyl Derivatives. *Journal of Pharmacology and Experimental Therapeutics*. 1999; 291(2):856–864. [PubMed: 10525109] (b) Shinotoh H, Inoue O, Suzuki K, Yamasaki T, Iyo M, Hashimoto K, Tominaga T, Itoh T, Tateno Y, Ikehira H. Kinetics of [11C]N,N-Dimethylphenylethylamine in Mice and Humans: Potential for Measurement of Brain MAO-B Activity. *Journal of Nuclear Medicine*. 1987; 28(6):1006–1011. [PubMed: 3495646] (c) Halldin C, Bjurling P, Stalnacke CG, Jossan SS, Orelund L, Langstrom B. 11C-labelling of dimethylphenethylamine in two different positions and biodistribution studies. *International journal of radiation applications and instrumentation Part A, Applied radiation and isotopes*. 1989; 40(7): 557–60.
8. Livni E, Spellman JP, Correia JA, Alpert NM, Brownell GL, Strauss HW, Elmaleh DR. [11C]MPTP: A Potential Tracer for Parkinson's Disease Research in Laboratory Animals. *Journal of Nuclear Medicine*. 1986; 27(10):1600–1603. [PubMed: 3489823]
9. (a) Efang SM, Mash D, Hefti F, Kung HF, Billings J. Selective visualization of rodent locus ceruleus by a radiolabeled N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine analog. *Journal of neurochemistry*. 1989; 53(2):459–64. [PubMed: 2787390] (b) Efang SM, Kung HF, Mash DC, Jabir M, Billings J, Pablo J, Dutta A, Freshler A. Pargyline-sensitive selective accumulation of a

- radiolabeled MPTP analog in the primate cerebral cortex and basal ganglia. *Synapse* (New York, NY). 1990; 5(3):207–12.
10. Zhao Z, Dalvie D, Naiman N, Castagnoli K, Castagnoli N. Design, synthesis, and biological evaluation of novel 4-substituted 1-methyl-1,2,3,6-tetrahydropyridine analogs of MPTP. *Journal of Medicinal Chemistry*. 1992; 35(23):4473–4478. [PubMed: 1447747]
 11. (a) Wang YX, Castagnoli N. Studies on the Monoamine Oxidase (MAO)-Catalyzed Oxidation of Phenyl-Substituted 1-Methyl-4-phenoxy-1,2,3,6-tetrahydropyridine Derivatives: Factors Contributing to MAO-A and MAO-B Selectivity. *Journal of Medicinal Chemistry*. 1995; 38(11): 1904–1910. [PubMed: 7783122] (b) Yu J, Castagnoli N Jr. Synthesis and MAO-B substrate properties of 1-methyl-4-heteroaryl-1,2,3,6-tetrahydropyridines. *Bioorganic & medicinal chemistry*. 1999; 7(2):231–9. [PubMed: 10218814]
 12. Kalgutkar AS, Castagnoli K, Hall A, Castagnoli N Jr. Novel 4-(aryloxy)tetrahydropyridine analogs of MPTP as monoamine oxidase A and B substrates. *J Med Chem*. 1994; 37(7):944–9. [PubMed: 8151621]
 13. Long S, Chen L, Xiang Y, Song M, Zheng Y, Zhu Q. An activity-based fluorogenic probe for sensitive and selective monoamine oxidase-B detection. *Chemical communications* (Cambridge, England). 2012; 48(57):7164–6.
 14. (a) Fowler JS, Ding YS, Logan J, MacGregor RR, Shea C, Garza V, Gimi R, Volkow ND, Wang GJ, Schlyer D, Ferrieri R, Gatley SJ, Alexoff D, Carter P, King P, Pappas N, Arnett CD. Species differences in [11C]clorgyline binding in brain. *Nuclear Medicine and Biology*. 2001; 28(7):779–785. [PubMed: 11578898] (b) Novaroli L, Daina A, Favre E, Bravo J, Carotti A, Leonetti F, Catto M, Carrupt PA, Reist M. Impact of species-dependent differences on screening, design, and development of MAO B inhibitors. *J Med Chem*. 2006; 49(21):6264–72. [PubMed: 17034132]
 15. (a) Riachi NJ, Harik SI. Monoamine oxidases of the brains and livers of macaque and cercopithecus monkeys. *Experimental neurology*. 1992; 115(2):212–7. [PubMed: 1735467] (b) Campbell IC, Marangos PJ, Parma A, Garrick NA, Murphy DL. Localization of monoamine oxidases A and B in primate brains relative to neuron-specific and non-neuronal enolases. *Neurochemical research*. 1982; 7(6):657–66. [PubMed: 7121716]
 16. Fowler JS, Volkow ND, Logan J, Wang GJ, MacGregor RR, Schlyer D, Wolf AP, Pappas N, Alexoff D, Shea C, et al. Slow recovery of human brain MAO B after L-deprenyl (Selegiline) withdrawal. *Synapse* (New York, NY). 1994; 18(2):86–93.
 17. Ottoboni S, Caldera P, Trevor A, Castagnoli N Jr. Deuterium isotope effect measurements on the interactions of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine with monoamine oxidase B. *The Journal of biological chemistry*. 1989; 264(23):13684–8. [PubMed: 2788162]

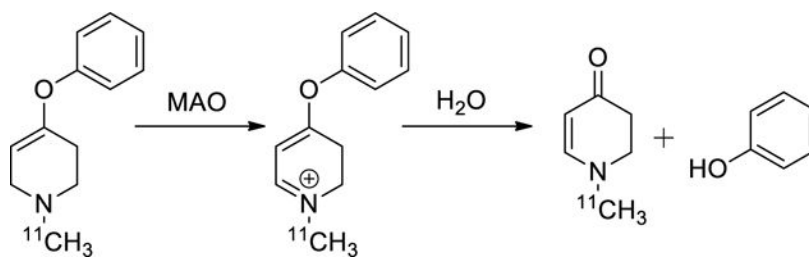


Figure 1. Monoamine oxidase-mediated oxidation of 1-[¹¹C]methyl-4-aryloxy-1,2,3,6-tetrahydropyridines, followed by spontaneous hydrolysis, yields a phenol and 1-[¹¹C]methyl-2,3-dihydro-4-pyridinone.

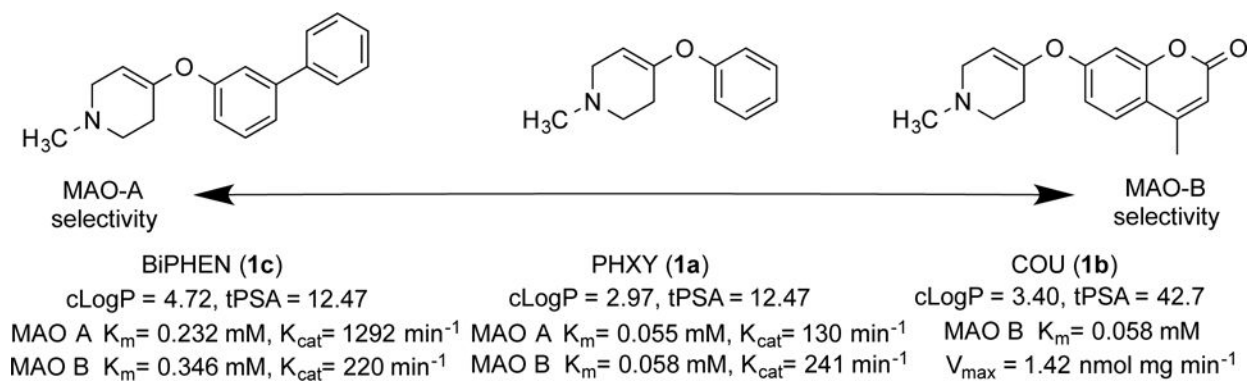


Figure 2.
Target carbon-11 radiotracers and reported in vitro enzyme kinetics.

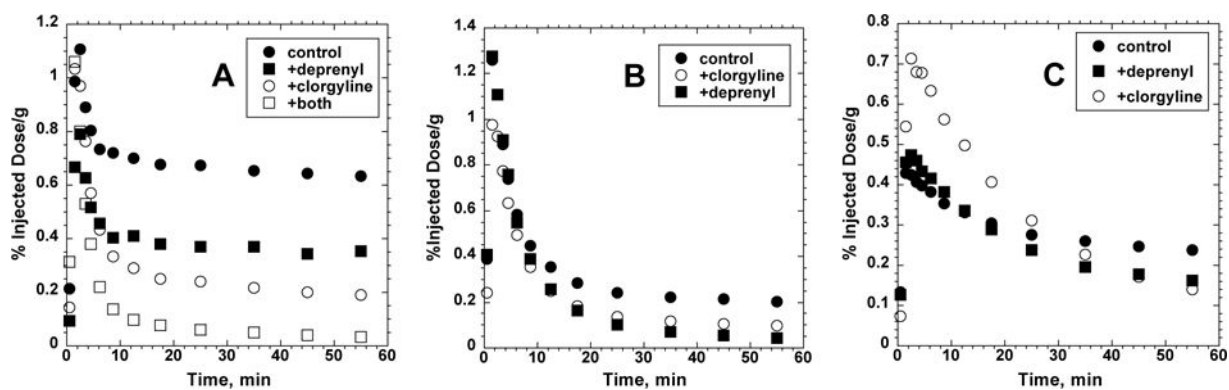


Fig. 3.

Representative curves (single scans) for *in vivo* trapping of $[^{11}\text{C}]\text{PHXY}$ (panel A), $[^{11}\text{C}]\text{COU}$ (panel B) and $[^{11}\text{C}]\text{BiPHEN}$ (panel C) in rat brain cerebrium. Data is expressed as percent injected dose per gram for rat brain cerebrium. Pharmacological interventions were done using pretreatment with MAO-A inhibitor (clorgyline) or MAO-B inhibitor (deprenyl) or both together.

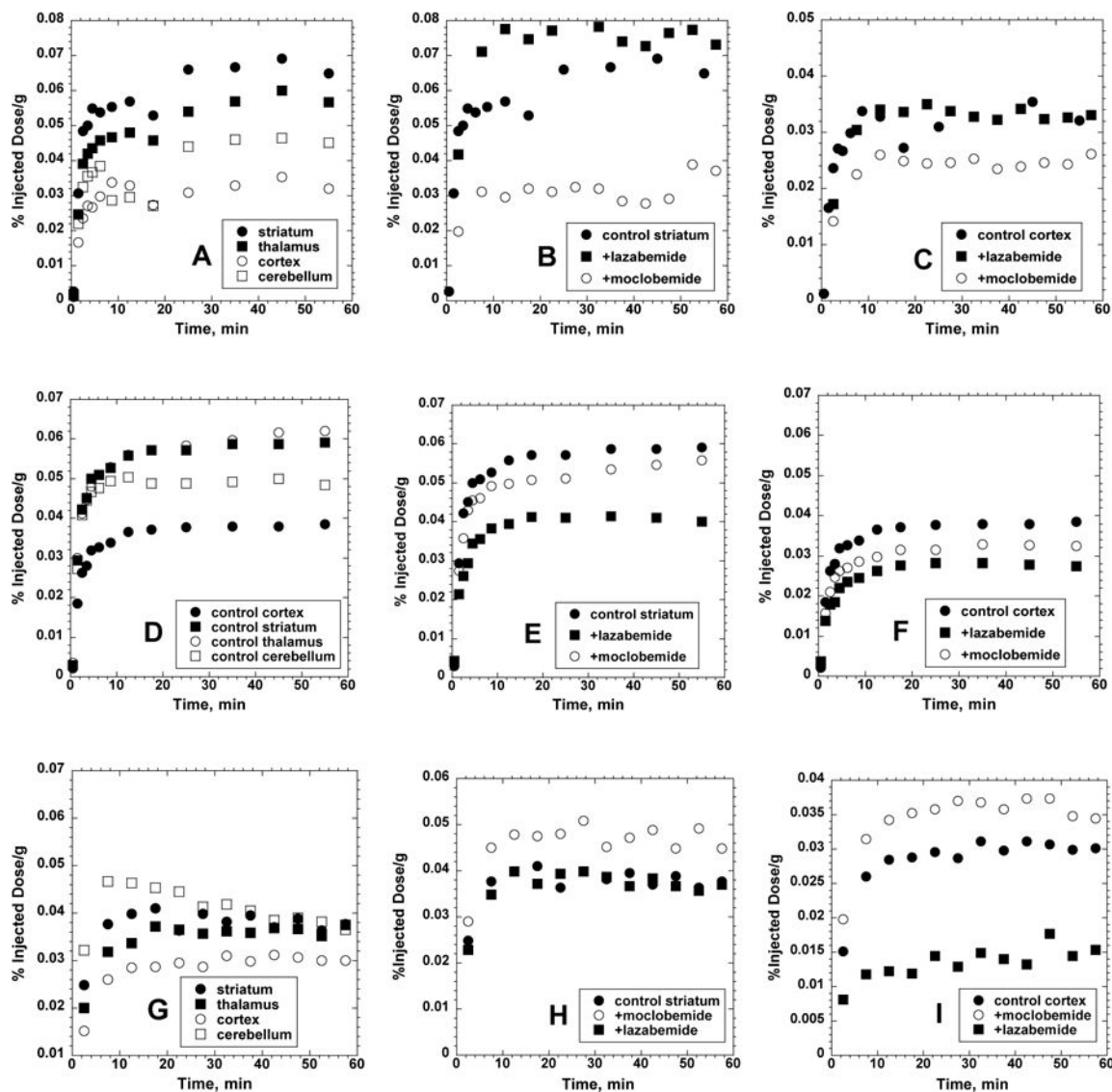
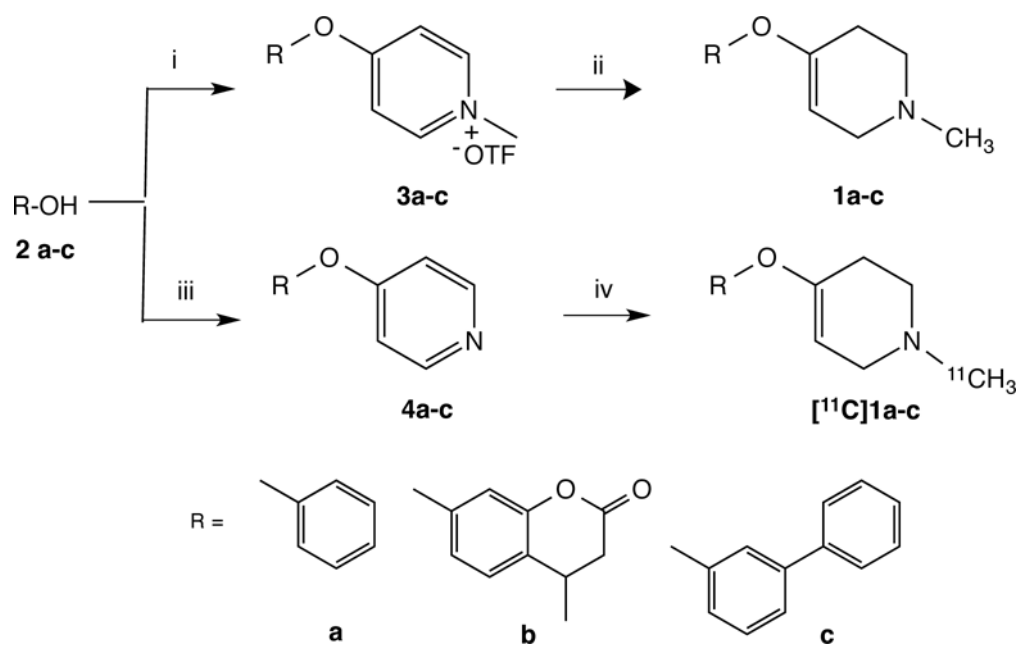


Fig. 4. Representative curves (single scans) for *in vivo* trapping of $[^{11}\text{C}]\text{PHXY}$ (panels A–C), $[^{11}\text{C}]\text{COU}$ (panel D–F) and $[^{11}\text{C}]\text{BiPHEN}$ (panels G–I) in striatum, thalamus, cerebral cortex and cerebellum of rhesus monkey brain. Pharmacological interventions were done using pretreatment with MAO-A inhibitor (moclobemide) or MAO-B inhibitor (lazabemide).

**Scheme 1.**

Syntheses of unlabeled and carbon-11 labeled tetrahydropyridine MAO substrates.^a

^aReagents and Conditions (i) NaOCH₃, DMF, 4-chloro-1-methylpyridin-1-ium triflate; (ii) NaBH₄, CH₃OH; (iii) KO^tBu, DMF, 4-chloropyridin-1-ium; (iv) [^{11}C]CH₃OTf, EtOH, NaBH₄