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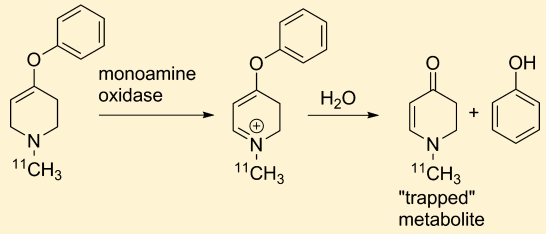
In Vivo Metabolic Trapping Radiotracers for Imaging Monoamine Oxidase-A and -B Enzymatic Activity

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

Supporting Information

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 to be 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-[^{11}C]methyl-4-aryloxy-1,2,3,6-tetrahydropyridines as metabolic trapping agents for the monoamine oxidases. MAO-mediated oxidation and spontaneous hydrolysis yield 1-[^{11}C]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 brains. 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-[^{11}C]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, 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 disease.[1] 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 interest.[2] The importance of the monoamine oxidases in numerous disease processes stimulated interest for *in vivo* noninvasive 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) studies.[3] Prior approaches to isoform-selective MAO radiotracer development have mostly concentrated on (a) irreversibly trapped suicide inhibitors (e.g., [^{11}C]deprenyl and [^{11}C]-clorgyline) that form covalent bonds between the radiotracer and the flavin cofactor of the enzymes and (b) reversibly binding high-affinity inhibitors (e.g., [^{11}C]befloxatone, [^{11}C]-harmine). Several of these have been successfully introduced into human PET studies, including evaluation in Alzheimer's disease,[4] 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 is still retained within the target tissue. This is the mechanism behind such *in vivo* imaging radiotracers as 2-[^{18}F]fluoro-2-deoxyglucose ([^{18}F]FDG, glucose metabolism), *N*-[^{11}C]-methyl-4-piperidinyl-propionate or acetate ([^{11}C]PMP and [^{11}C]MP4A, acetylcholinesterase),[6] and [^{18}F]fluoroDOPA (dopamine synthesis), among other examples. The concept of noncovalent 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 were reported in rodents, and even a single human PET image was presented.[7] Preliminary studies in both rat and primate were also done with carbon-11-labeled MPTP[8] and a radioiodinated tetrahydropyridine (*N*-methyl-4-(4′-hydroxy-3′-[^{125}I]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 anal-

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yses) of noncovalent 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 (Figure 1), forming dihydropyridinium species that are then

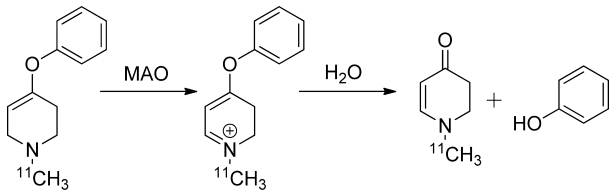


Figure 1. Monoamine oxidase-mediated oxidation of 1-[^{11}C]methyl-4-aryloxy-1,2,3,6-tetrahydropyridines, followed by spontaneous hydrolysis, yields a phenol and 1-[^{11}C]methyl-2,3-dihydro-4-pyridinone.

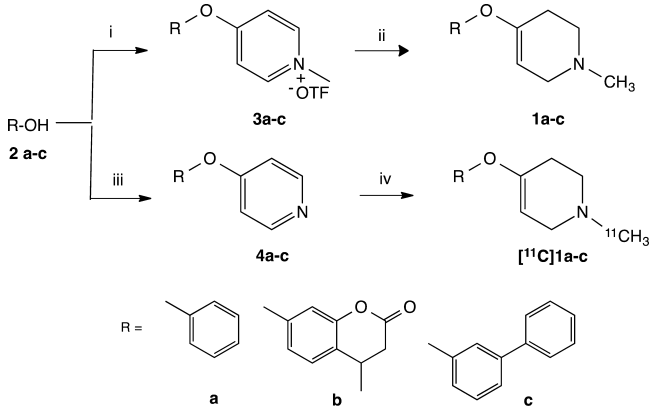
rapidly hydrolyzed to a phenol and 1-methyl-2,3-dihydro-4-pyridinone.[10] As that ketone is hydrophilic (cLog P = −0.18), labeling with a carbon-11 at the N-methyl substituent was hypothesized to provide a metabolite that would be trapped within brain tissues. Furthermore, as a second oxidation step of the dihydropyridinium intermediate never occurs, the 4-aryloxy-1,2,3,6-tetrahydropyridines are nontoxic (in contrast to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)). A series of 4-aryloxy-substituted tetrahydropyridines was synthesized by Castagnoli and co-workers[11] 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 is 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 (Figure 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 toward MAO-B.[12] The biphenyl-substituted derivative (BiPHEN, **1c**) demonstrated 8-fold higher *in vitro* reactivity toward MAO-A over MAO-B (Wang and Castagnoli, 1995).[11a] Finally, the 7-coumarinyl derivative (COU, **1b**) was reported by Long and co-workers[13] 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-aryloxypyridines (**3a−c**), followed by sodium borohydride reduction of the pyridine ring (Scheme 1). Products **1a−c** were

Scheme 1. Syntheses of Unlabeled and Carbon-11-Labeled Tetrahydropyridine MAO Substrates[a]



[a]Reagents and conditions: (i) $NaOCH_3$, DMF, 4-chloro-1-methylpyridin-1-ium triflate; (ii) $NaBH_4$, CH_3OH; (iii) KO*t*Bu, DMF, 4-chloropyridin-1-ium; (iv) [^{11}C]CH_3OTf, EtOH, $NaBH_4$.

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-[^{11}C]methylation of the 4-aryloxypyridines **4a−c** using no-carrier-added [^{11}C]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 corresponding phenols **2a−c**. The radiotracers ([^{11}C]PHXY (**1a**), [^{11}C]COU (**1b**), and [^{11}C]BiPHEN (**1c**)) were isolated and purified by HPLC, with overall synthesis times of 30 min. Although their 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 oxidation by the monoamine oxidases,[7a,14] additional studies were performed in the rhesus monkey.

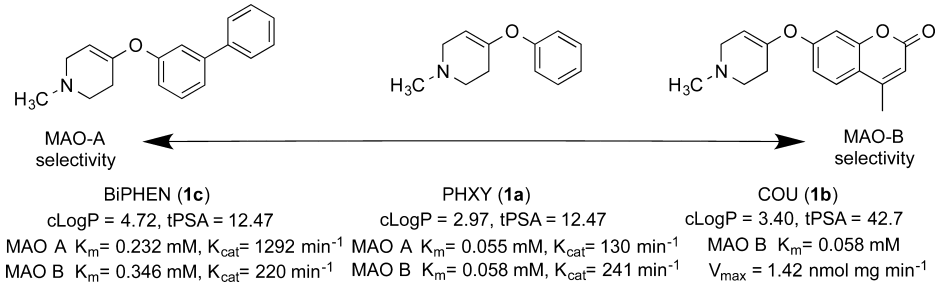


Figure 2. Target carbon-11 radiotracers and reported *in vitro* enzyme kinetics.

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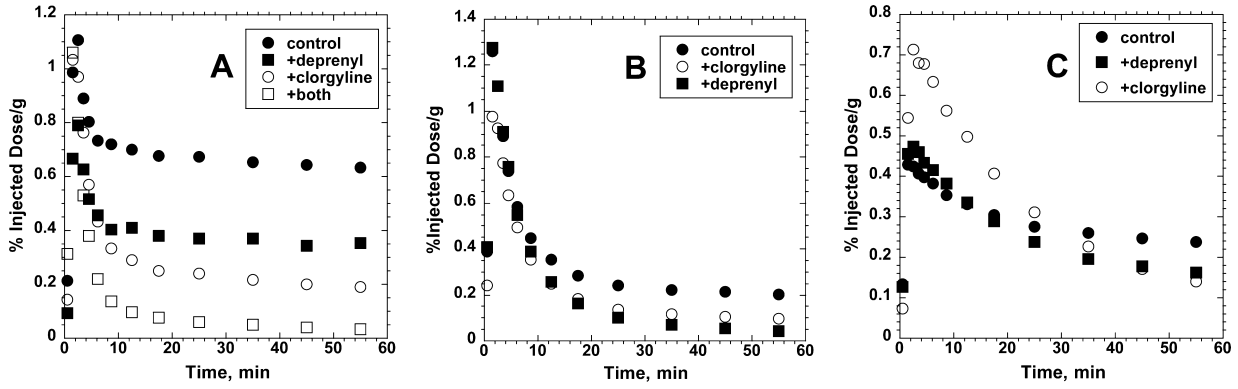

Figure 3. Representative curves (single scans) for *in vivo* trapping of [^{11}C]PHXY (A), [^{11}C]COU (B), and [^{11}C]BiPHEN (C) in rat brain cerebrum. Data is expressed as percent injected dose per gram for rat brain cerebrum. Pharmacological interventions were done using pretreatment with MAO-A inhibitor (clorgyline), MAO-B inhibitor (deprenyl), or both together.

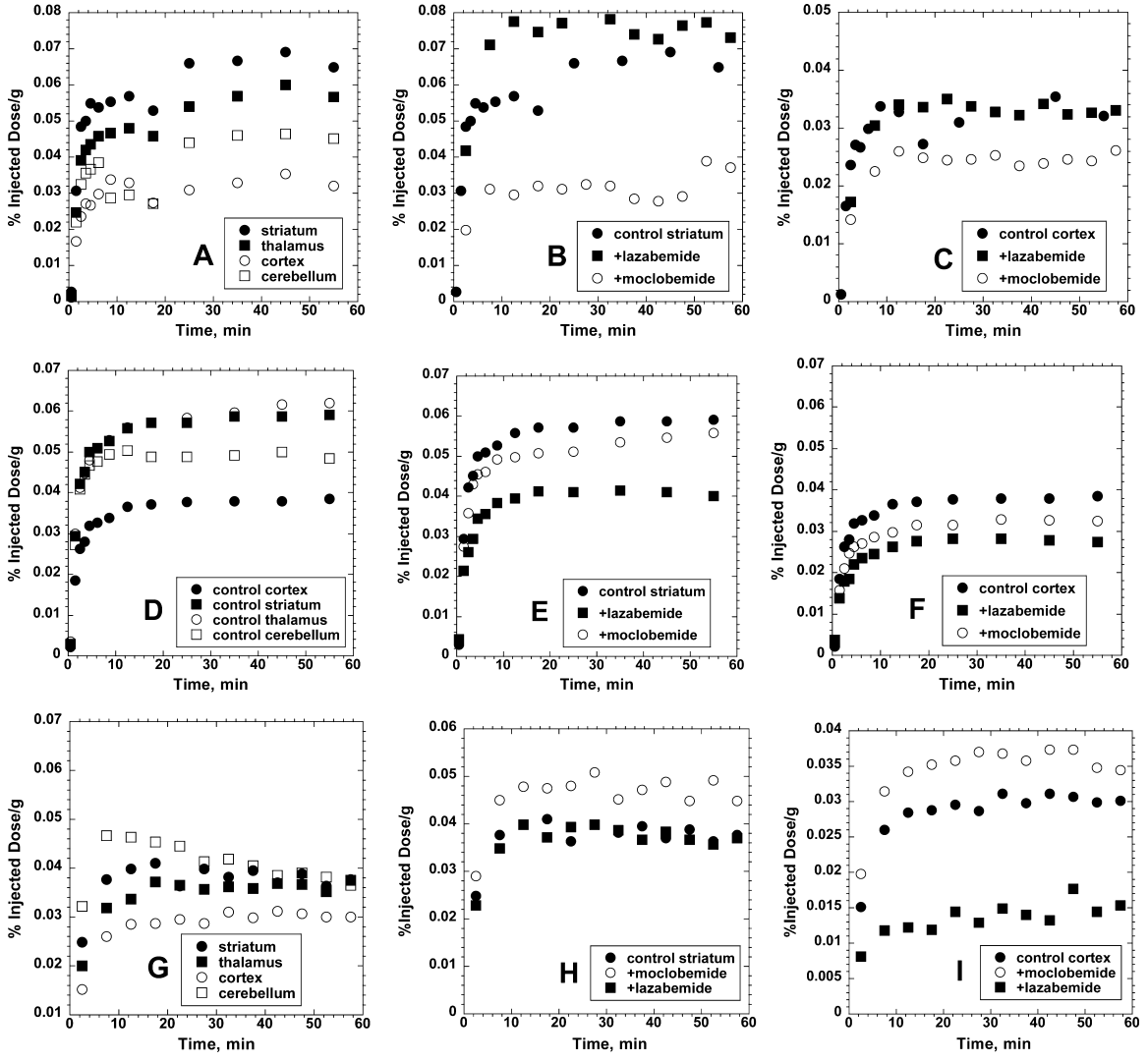

Figure 4. Representative curves (single scans) for *in vivo* trapping of [^{11}C]PHXY (A–C), [^{11}C]COU (D–F), and [^{11}C]BiPHEN (G–I) in striatum, thalamus, cerebral cortex, and cerebellum of rhesus monkey brain. Pharmacological interventions were done using pretreatment with MAO-A inhibitor (moclobemide)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

The microPET studies in rat brain demonstrated similar pharmacokinetics for [^{11}C]PHXY and [^{11}C]COU, with rapid initial uptakes peaking at 90–150 s and equivalent maximum brain concentrations (1.1% injected dose/g for both). For both [^{11}C]PHXY and [^{11}C]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 [^{11}C]PHXY and 10–15% for [^{11}C]COU.

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[^{11}C]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), clorgyline (selective MAO-A inhibitor, 10 mg/kg i.p. 90 min prior to scan), or both irreversible inhibitors. The *in vivo* trapping of [^{11}C]PHXY and [^{11}C]BiPHEN was more sensitive to MAO-A inhibition, and [^{11}C]COU was more sensitive to MAO-B inhibition, but none of the three radiotracers examined here exhibited specificity for either isozyme in the rat brain (Figure 3). The pharmacological blocking studies support little nonspecific binding of [^{11}C]PHXY and [^{11}C]COU, as residual trapped radioactivity levels are very low after enzyme inhibition. The lower brain uptake and slower kinetics of [^{11}C]BiPHEN suggest that the radiotracer might exhibit higher nonspecific 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 min after injection of [^{11}C]PHXY confirmed the formation of a single polar radioactive metabolite, consistent with formation of 1-[^{11}C]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 toward 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 that seen in the rat brain, with more encouraging isozyme selectivity for [^{11}C]PHXY and [^{11}C]COU. [^{11}C]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) (Figure 4A). The coumarin derivative, [^{11}C]COU, showed similar pharmacokinetics, with slightly lower trapping of radioactivity and little or no distinction between levels in the striatum and thalamus (Figure 4D). Finally, the kinetics of [^{11}C]BiPHEN was significantly different, with much lower brain uptake and trapping and less heterogeneity in the regional brain distribution (Figure 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 brain.[15] The rank order of striatum > cerebellar cortex > cortex for [^{11}C]COU is, however, consistent with the *in vitro* studies of Riachi and Harik[15a] using either binding of [^{3}H]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 clorgyline.[16] 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 to be pharmacological intervention studies but are not necessarily full-blocking studies (in the sense the enzymes can be fully and irreversibly inhibited, as was achieved with deprenyl and clogyline 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 Figure 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 that 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 (Figure 4E,F), with lesser effects effects for inhibition of MAO-A. Again, the monkey imaging studies are more encouraging than those of the rat, where significant effects 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 that 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 (Figure 4H,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 nonspecific distribution due to its high lipophilicity (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

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general synthetic strategy shown in Scheme 1 can be used to prepare a wide variety of substituted tetrahydropyridines in the continued search for radiotracers that have the optimal combination 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 was demonstrated to be effective for MPTP.[17]

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 was reflected in the relatively little selectivity of [^{11}C]PHXY and [^{11}C]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 clogyline) that unequivocally block enzyme action poses a challenge given the now recognized long-lasting effects of such inhibitors on the concentrations of active enzyme molecules.[16]

METHODS

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 the 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 the mixture was stirred for 10 min. 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 $NaBH_4$ (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 (3×), 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-phenoxypyridine, the reduction step reaction yielded 0.056 g (41% yield) of **1a** as a white solid. ^{1}H NMR (400 MHz; CH_3OD-d_4)/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 calcd for $[M + H]^+$ ($M = C_{12}H_{15}NO$), 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. ^{1}H NMR (400 MHz; CH_3OD-d_4)/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 calcd for $[M + H]^+$ ($M = C_{16}H_{17}NO_3$), 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. ^{1}H NMR (400 MHz; CH_3OD-d_4)/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 calcd for $[M + H]^+$ ($M = C_{18}H_{19}NO$), 266.1539; found, 266.1538.

*General Procedure for the Preparation of 4-Aryloxypyridines (**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 at 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 NH_4Cl. The product was extracted with ethyl acetate (3×), 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. ^{1}H NMR (400 MHz; CH_3OD-d_4)/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 calcd for $[M + H]^+$ ($M = C_{15}H_{11}NO_3$), 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. ^{1}H NMR (400 MHz; CH_3OD-d_4)/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 calcd for $[M + H]^+$ ($M = C_{17}H_{13}NO$), 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, and 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 $FX_{C\text{-}Pro}$ automated radiochemistry synthesis module (General Electric, GE). [^{11}C]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 ([^{11}C]CH_3OTf). The [^{11}C]CH_3OTf in helium carrier gas was bubbled into a vial containing a solution of precursor (1 mg) dissolved in ethanol (0.2 mL). At the 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 semipreparative HPLC loop. The product was purified by reverse-phase chromatography (Prodigy ODS prep, 250 × 10 mm, 10 μ, 4 mL/min), collected, diluted into H_2O (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 nonhuman primate studies. Average specific activity was 69 116 GBq/mmol (range of 23 236 to 178 081 GBq/mmol). Overall synthesis times were 30 min from end-of-bombardment.

*[^{11}C]1-Methyl-4-phenoxy-1,2,3,6-tetrahydropyridine ([^{11}C]PHXY, [^{11}C]**1a**).* In nonoptimized yields, 270 ± 173 mBq of the phenyl ether (0.8% yield from [^{11}C]CH_3OTf, not decay corrected; n = 8) was

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collected. The product was purified by semipreparative reverse-phase chromatography (250 × 10, 10 μ, ODS prep column eluted with 40% CH_3CN, 60% H_2O, 10 mM NH_4OAc).

*[^{11}C]4-Methyl-7-(pyridin-4-yloxy)-2H-chromen-2-one ([^{11}C]COU, [^{11}C]**1b**).* In nonoptimized yields, 577 ± 141 MBq of the coumarin ether (1.7% yield from [^{11}C]CH_3OTf, not decay corrected; n = 10) was collected. The product was purified by semipreparative reverse-phase chromatography (250 × 10, 10 μ, ODS prep column eluted with 30% CH_3CN, 70% H_2O, 10 mM NH_4OAc).

*[^{11}C]4-([1,1′-Biphenyl]-3-yloxy)-1-methyl-1,2,3,6-tetrahydropyridine ([^{11}C]BiPHEN, [^{11}C]**1c**).* In nonoptimized yields, 551 ± 126 MBq of the biphenyl ether (1.7% yield from [^{11}C]CH_3OTf, nondecay corrected; n = 7) was collected. The product was purified by semipreparative reverse-phase chromatography (250 × 10, 10 μ, ODS prep column eluted with 50% CH_3CN, 50% H_2O, 10 mM NH_4OAc, pH 4.5).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.5b00223.

Spectra for all novel compounds synthesized, chromatographic data for radiotracer purifications, and procedures for microPET imaging (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*(P.J.H.S.) E-mail: pjhscott@umich.edu. Tel.: +1 (734) 615-1756.

*(M.R.K.) E-mail: mkilbour@umich.edu. Tel.: +1 (734) 763-9246.

Author Contributions

M.R.K., P.J.H.S., and A.F.B. contributed to design of experiments, analysis of data, and composition of the manuscript. A.F.B., X.S., C.A.S., and P.S. performed the experiments.

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Notes

The authors declare no competing financial interest.

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