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FULL PAPER

[*Carboxyl*–^{11}C]-Labeling of four high-affinity cPLA2α inhibitors and their evaluation as radioligands in mice with positron emission tomography

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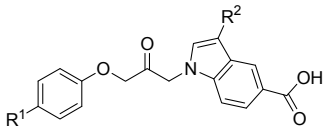
Abstract: Cytosolic phospholipase A2α (cPLA2α) may play a critical role in neuropsychiatric and neurodegenerative disorders associated with oxidative stress and neuroinflammation. An effective PET radioligand for imaging cPLA2α in living brain might prove useful for biomedical research, especially on neuroinflammation. We selected four high-affinity (IC_{50} 2.1 to 12 nM) indole-5-carboxylic acid-based inhibitors of cPLA2α, namely 3-isobutyryl-1-(2-oxo-3-(4-phenoxyphenoxy)propyl)-1*H*-indole-5-carboxylic acid (**1**); 3-acetyl-1-(2-oxo-3-(4-(4-(trifluoromethyl)-phenoxy)phenoxy)propyl)-1*H*-indole-5-carboxylic acid (**2**); 3-(3-methyl-1,2,4-oxadiazol-5-yl)-1-(2-oxo-3-(4-phenoxyphenoxy)propyl)-1*H*-indole-5-carboxylic acid (**3**); and 3-(3-methyl-1,2,4-oxadiazol-5-yl)-1-(3-(4-octylphenoxy)-2-oxopropyl)-1*H*-indole-5-carboxylic acid (**4**), for labelling in carboxyl position with carbon-11 ($t_{1/2}$ = 20.4 min) to provide candidate PET radioligands for imaging brain cPLA2α. [^{11}C]**1**–**4** were obtained for intravenous injection in adequate overall yields (1.1−5.5%) from cyclotron-produced [^{11}C]carbon dioxide and with moderate molar activities (70−141 GBq/μmol) through the use of Pd(0)-mediated [^{11}C]carbon monoxide insertion on iodo precursors. Measured log$D_{7.4}$ values were within a narrow moderate range (1.9−2.4). After intravenous injection of [^{11}C]**1**–**4** in mice, radioactivity uptakes in brain peaked at low values (≤ 0.8 SUV) and decreased by about 90% over 15 min. Pre-treatments of the mice with high doses of the corresponding non-radioactive ligands did not alter brain time-activity curves. Brain uptakes of radioactivity after administration of [^{11}C]**1** to wild type and P-gp/BCRP dual knock-out mice were similar (peak 0.4 *vs*. 0.5 SUV), indicating that [^{11}C]**1** and others in this structural class, are not efflux transporters substrates.

Introduction

Cytosolic phospholipase A2α (cPLA2α) is one of the six members of group IV phospholipase A2 (GIVA PLA2) enzyme family.[1-5] In the arachidonic acid cascade, cPLA2α is activated by increases in intracellular Ca^{2+} and kinase activation as a result of extracellular stimulation, and hydrolyses phospholipids to arachidonic acid and lysophospholipids in brain.[2,6] Arachidonic acid is metabolized through the cyclooxygenase (COX) and lipoxygenase (LOX) pathways to yield prostaglandins and leukotrienes. These eicosanoids promote neuroinflammation through increased vascular permeability and the recruitment of granulocytes such as neutrophils to the site of injury or infection. Increased cPLA2α activity and excessive production of proinflammatory mediators, eicosanoids, and platelet activating factor, may lead to neurodegenerative and neuropsychiatric disease states, such as Alzheimer's disease, Parkinson's disease, and traumatic brain injury.[7] Thus, cPLA2α is potentially an attractive biomarker for quantifying disease and for designing drugs to treat neuroinflammation and oxidative stress associated disorders. The development of PET radioligands that specifically target cPLA2α could help to evaluate and understand the role of this enzyme in the pathophysiology of human diseases and would be complementary to efforts to develop PET radioligands for other neuroinflammation targets,[8] such as translocator 18 kDa protein (TSPO),[8-10] COXs,[8,11,12] P2X purinoceptor 7 ($P2X_7$),[8,13] and cannabinoid receptor subtype 2 (CB_2).[8,14] So far, only [^{11}C]arachidonic acid has been used with PET for pre-clinical and clinical imaging[15,16] to explore brain cPLA2 activity.[17] However, because of the involvement of arachidonic acid in neural functions other than signal transduction, such as membrane synthesis and remodelling, the incorporation rate (*K**) has not been deemed entirely specific to the cPLA2 signalling system.[17]



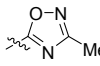
	R^1	R^2	
1	PhO		(CO^{i}Pr)
2	4-CF_3PhO		(COMe)
3	PhO		(3-Me-1,2,4-oxadiazole)
4	*n*-C_8H_{17}		

Figure 1. Structures of **1**–**4**.

Many selective inhibitors of cPLA2α are known.[3,4,18] We choose a small group of indole-5-carboxylic acids[19,20] (**1**–**4**, **Figure 1**) for PET radioligand development based on properties that are suitable for this purpose, such as adequately low molecular weight (< 500), moderate polar surface area, wide log*P* range (2.6–6.3) and potent enzyme inhibitory ability (in the low nM range).[21]

The importance of the carboxyl group as a component of the pharmacophore needed for potent cPLA2α inhibitory activity is highlighted by the lower potency seen when this group is replaced with bioisosteres, such as hydroxamic acid, tetrazole, or acyl-cyanamide.[22] cPLA2α strictly recognizes the "carboxyl group" through interaction with the serine-228 residue of the active site. Thus, 'first generation' radioligands for cPLA2α are likely to contain a carboxyl group. Because of extensive ionization at physiological pH, [^{11}C]carboxylic acids (pK_a ~ 4) do not generally cross the blood-brain barrier (BBB), but some, such as [^{11}C]arachidonic acid (clog*D*, 4.89) do enter brain, even if only to a limited extent.[17,23,24] In this study, the carboxyl group also provided a generic site for labelling with carbon-11 ($t_{1/2}$ = 20.4 min). Here, we describe the labelling of **1**−**4** in their carboxyl groups, for preliminary evaluation as PET radioligands for cPLA2α in mice.

Results and Discussion

Generally, [*carbonyl*–^{11}C]arylcarboxylic acids have been synthesized from aryl halides, aryliodonium salts, arylboronic acids, or arylboronic esters with either [^{11}C]carbon monoxide or [^{11}C]carbon dioxide.[25-28] However, some methods suffer drawbacks, such as reagent air/moisture sensitivity,[26] requirement to use high amounts of precursor,[25] or low molar activity.[29] [^{11}C]Carbon monoxide has been gaining importance as a useful primary labelling agent because: i) it can be synthesised by passage of cyclotron-produced [^{11}C]carbon dioxide over heated molybdenum (875 °C) in high-yield and high molar activity;[30] (ii) new apparatus[31-36] and techniques[37-41] have been developed for increasing [^{11}C]carbon monoxide availability in small volumes of organic solvents; (iii) new methods for the transition-metal mediated insertions of [^{11}C]carbon monoxide into radiotracers;[42-47] have expanded choice of reagents and functional group tolerance. We chose to explore the Pd(0)-mediated [^{11}C]carbon monoxide insertion in iodo precursors for the labeling of **1**−**4** because this method requires low amounts of iodo precursors that should be stable and air-insensitive, and because the method is expected to show high functional group tolerance. In addition, we had access to an autoclave for performing such reactions and experience with its use.[48,49]

Precursor synthesis

All iodo precursors for labeling were synthesised from commercially available 5-iodoindole (**Figure 2**). Direct acylation of 5-iodoindole with acetyl chloride or isobutyryl chloride gave the methyl substituted (**5**) or isopropyl substituted (**6**) indole ketone, respectively, in high and moderate yield, respectively. **5** and **6** were subsequently converted into the epoxides **9** and **10**, respectively, by treatment with neat epichlorohydrin in the presence of KOH and tetrabutylammonium bromide. Reaction of 5-iodoindole with trichloroacetyl chloride in the presence of pyridine, followed by methanolysis produced the methyl ester **7**. Condensation of **7** with *N*-hydroxyacetamidine in THF gave **8**, and subsequent reaction with neat epichlorohydrin in the presence of KOH and tetrabutylammonium bromide afforded the epoxide **11** (Panel A, **Figure 2**). Iodo precursors for labeling (**16**–**19**) were prepared by treating an appropriate epoxide (**9**–**11**) intermediate with a corresponding substituted phenol in the presence of DMAP to obtain the intermediate alcohols **12**–**15**, followed by oxidation with Dess-Martin periodinane reagent (Panel B, **Figure 2**). Only the yields for compounds having the *n*-octyl chain (**15** or **19**) were low. No further optimization was attempted because the quantities of the final iodo-compounds were sufficient for several labeling experiments.

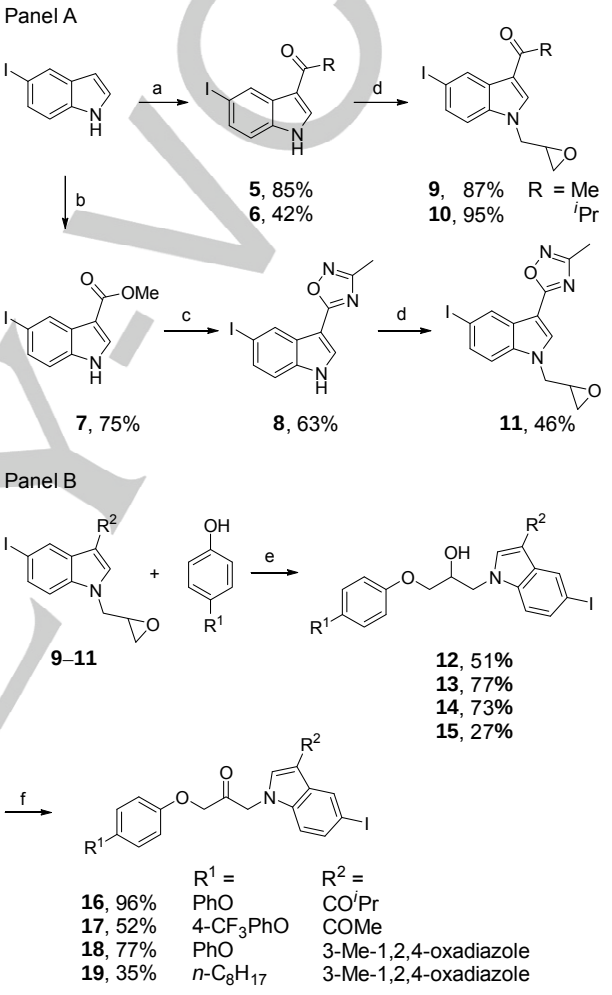


Figure 2. Syntheses of iodo precursors for radiolabelling **16**–**19**. Conditions: a) acetyl chloride or isobutyryl chloride, $AlCl_3$, DCM, RT, 8 h. b) (1) pyridine, trichloroacetyl chloride, dioxane, 80 °C, 2.5 h; (2) MeOH, NaOH, 80 °C, 30 min. c) *N*-hydroxyacetamidine, NaH, THF, RT, 1 h. d) epichlorohydrin, KOH, TBAB, RT, 1 h. e) ArOH, DMAP, DCM, 120 °C, 1 h. f) Dess-Martin periodinane, DCM, RT, 4 h.

Radiochemistry

Reaction of **16** with [^{11}C]carbon monoxide in THF in the presence of $Pd(PPh_3)_4$ afforded [^{11}C]**1** in useful yields (**Figure 3**). The bright yellow color of $Pd(PPh_3)_4$ turned to brown over a period of several weeks due to air and light sensitivity. [^{11}C]Carbon monoxide insertion efficiency decreased over this period when conducted

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with the same batch of $Pd(PPh_3)_4$, despite storage in a nitrogen-protected glove-box. Attempts to use more air/moisture stable Pd reagents, such as $Pd(OAc)_2$ or $PdCl_2$ with Xantphos, for labeling reactions gave low yields. Efficiency of incorporation of [^{11}C]carbon monoxide could be restored by using fresh $Pd(PPh_3)_4$ or a slightly increased amount (5–10% mol) of slightly discolored batch. Subsequently $Pd(PPh_3)_4$ in THF was used for other labeling reactions. [^{11}C]**1**–**4** were obtained as formulated doses for intravenous injection in useful yields (1.1–5.5% from [^{11}C]carbon dioxide) and with moderate molar activities (70–141 GBq/μmol) (**Table 1**). Radiochemical purities exceeded 95%. The identity of ^{11}C-labeled radioligands was verified by co-injection of reference compounds on analytical HPLC and by LC-MS analysis of the carrier. Total synthesis time was around 45 min. Since the quantities were adequate for small animal microPET imaging studies, radiochemistry was not further optimized.

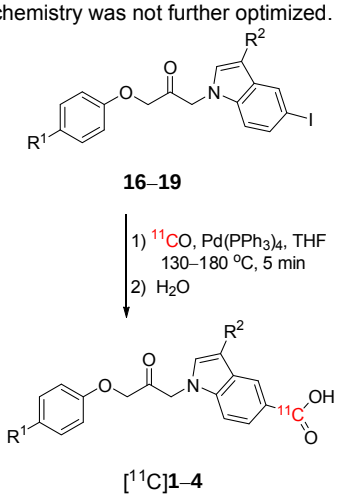

Figure 3. Radiosynthesis of [^{11}C]**1–4** through Pd(0)-mediated ^{11}C-carbonylation of corresponding iodo precursors.

Table 1. Radiosynthesis parameters.

Radioligand	Prep. HPLC method[a]	t_R (min)	Yield[b] (%)	Molar activity (GBq/μmol)
[^{11}C]**1**	I	9.5	1.7	114 *(n = 5)*
[^{11}C]**2**	I	10.3	3.1	141 *(n = 2)*
[^{11}C]**3**	I	9.0	1.1	109 *(n = 3)*
[^{11}C]**4**	II	12.5	5.5	70 *(n = 2)*

[a] Preparative HPLC methods I and II are described in the Experimental section. [b] Yields are from cyclotron-produced [^{11}C]carbon dioxide.

In vitro measurements

In vitro experiments confirmed that [^{11}C]**1–4** possessed properties considered favourable for brain PET radioligands (**Table 2**). Measured log$D_{7.4}$ values were within the range usually regarded as desirable for brain radioligands,[21] and were consistently lower than the calculated log*P* values because of the ionization of the carboxyl groups.[19,20]

The values of plasma free fraction (f_P) in pooled human plasma were generally low, and especially for [^{11}C]**4** (**Table 2**). Although higher plasma free fractions would be desirable, such low free fractions are known among effective PET radioligands (e.g., [^{11}C]MePPEP).[50] Radioligand stabilities in sodium phosphate buffer (0.15 M) at pH 7.4 were excellent, except for [^{11}C]**4**. Stabilities in rat brain homogenates were also excellent. In vitro analysis showed that [^{11}C]**1** was stable in rat and monkey whole blood.

Table 2. Radioligand *in vitro* properties.

Radioligand	IC_{50}[a] (nM)	log*D*	f_P (%)	Stability in buffer (%)	Stability in rat brain homogenate (%)
[^{11}C]**1**	12	2.10 ± 0.01	1.46 ± 0.01	98.9 ± 0.2	96.9
[^{11}C]**2**	10	2.43 ± 0.01	1.24 ± 0.01	98.4 ± 0.2	97.5
[^{11}C]**3**	6.1	2.07 ± 0.02	n.d.[b]	99.7 ± 0.2	n.d.
[^{11}C]**4**	2.1	1.94 ± 0.15	0.072 ± 0.001	80.3 ± 3.4	104

[a] Data from references [19] and [20]. [b] n.d. = not determined.

Distribution of [^{11}C]**1** into cellular blood elements was affected by plasma protein. More than 94% of [^{11}C]**1** was bound to blood cells in the absence of autologous plasma, but the bound percentage was considerably less in the presence of plasma (**Figure 4**). The cellular blood uptake of [^{11}C]**1** may represent its possible interaction with cPLA2α in peripheral blood cells.[51]

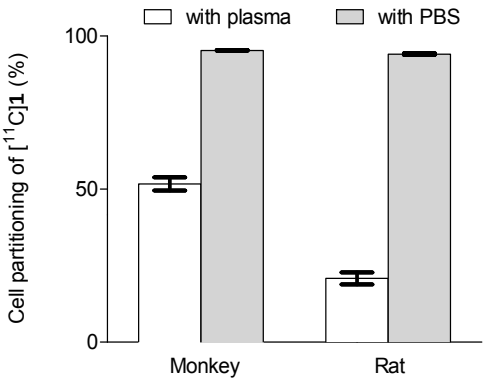

Figure 4. Distribution of [^{11}C]**1** in monkey and rat blood cells with or without plasma. Values are mean ± SD for n = 3.

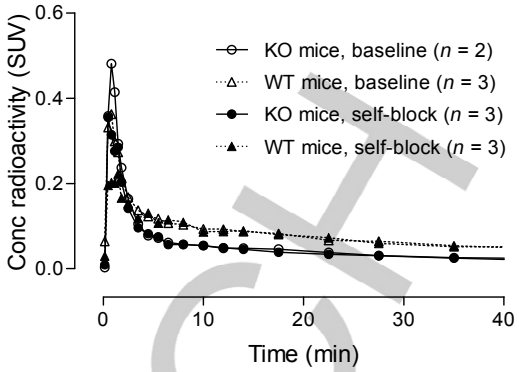

Figure 6. Brain time-activity curves for [^{11}C]**1** in wild type (WT) or efflux transporter knock-out (KO) mice under baseline and self-block conditions.

PET imaging

The measured moderate lipophilicities (log*D* values) of the four radioligands might suggest that they should be able to cross the blood-brain barrier. However, in wild type mice after intravenous administration of [^{11}C]**1**−**4**, peak radioactivities in brain were low (≤ 0.8 SUV) and declined by > 90% within 15 min (**Figure 5**). Ligand pretreatment with **1** at 2 mg/kg in wild type mice did not alter the shapes of brain time-activity curve for [^{11}C]**1** (**Figure 6**), and therefore provided no evidence for specific binding of the radioligand to cPLA2α. The other radioligands, [^{11}C]**2**–**3**, were also tested in this manner. The shapes of the resulting time-activity curves were similar to those under baseline conditions and again were not suggestive of the presence of specific binding.

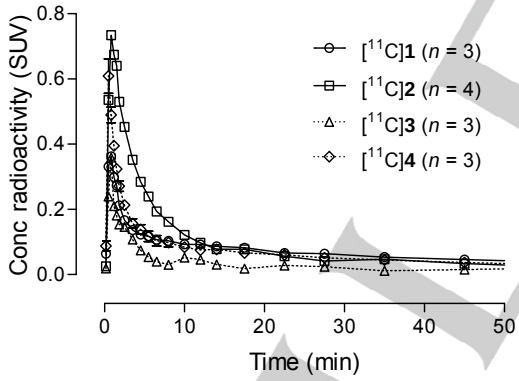

Figure 5. Brain time-activity curves for [^{11}C]**1**–**4** in wild type mice.

A primary factor underlying the lack of brain uptake of [^{11}C]**1**−**4** is likely the extensive ionization of the carboxyl groups to negatively charged carboxylate groups at physiological pH. A possibility is that ligand lipophilicity must be increased even further to allow brain entry. For example [^{11}C]arachidonic acid, which does get into brain to a low extent, has a high clog*D* value of 4.9.

Another possibility is that [^{11}C]**1**−**4** were excluded from brain by efflux transporters, such as P-glycoprotein (P-gp) or breast cancer resistance protein (BCRP).[52] Brain uptakes of [^{11}C]**1** in wild type and dual P-gp/BCRP knock-out mice were similar (peak SUV 0.4 *vs*. 0.5, **Figure 6**) under baseline or self-block conditions. Lack of brain penetration was therefore not due to action of these efflux transporters.

Peripheral organs are known to express higher concentrations of cPLA2α than brain.[51,53-55] **Figure 7** shows the time-activity curves for [^{11}C]**2** in these organs under baseline conditions. Much higher peak radioactivity was observed in peripheral organs, such as heart (5.6 SUV), lungs (2.9 SUV) or kidneys (1.7 SUV) for all four radioligands. No apparent blocking effects were observed when **2** was pre-administered at 2 mg/kg. These patterns of lacking preblocking effects were also seen with the other three radioligands injected into mice under baseline and self-blocking conditions.

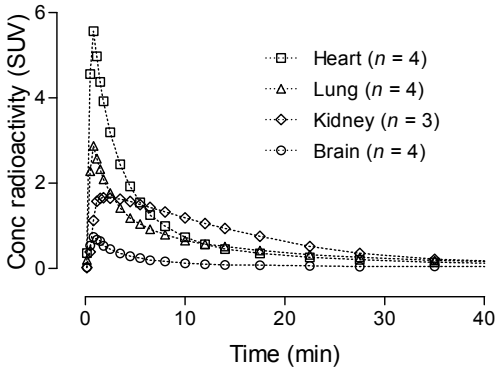

Figure 7. Time-activity curves for [^{11}C]**2** in different organs of wild type mice under baseline conditions.

It is also difficult at this stage to ascertain whether the low uptake and fast wash-out may also be attributed to the lack of cPLA2α enzyme density (B_{max}) in mouse brain, the insufficient radioligand affinity or other confounding factors.[21,52]

Conclusions

The Pd(0)-mediated ^{11}C-carbonylation of aryl iodides proved effevtive for producing structurally complex [^{11}C]arylcarboxylic acids. All tested radioligands [^{11}C]**1**–**4** showed low brain penetration and lack of retention in mouse brain *in vivo*. [^{11}C]**1** is not a brain P-gp or BRCP efflux transporter substrate. The results show that [^{11}C]**1**–**4** are ineffective brain PET radioligands for cPLA2α. They also provide no cPLA2α-specific binding in periphery. Successful PET imaging of brain cPLA2α remains desirable, but will likely require radioligands with no carboxyl group, higher brain penetration and higher enzyme affinity – a considerable medicinal chemistry challenge. Better animal models of cPLA2α activated inflammation with deep understanding of the underlying biology are also needed.

Experimental Section

Materials and Methods

All radiochemistry was performed in a lead-shielded hot-cell for radiation safety to personnel. Radioactivity was measured with a calibrated dose calibrator (Atomlab 300, Biodex Medical Systems, Shirley, NY) or a γ-counter (Wallac Wizard 3", 1480 automatic γ-counter; PerkinElmer, Waltham, MA). Radioactivity measurements were corrected for physical decay. Radiochemical yields are calculated for formulated radioligand from [^{11}C]carbon dioxide. Reagents and solvents are used as purchased unless otherwise specified.

Syntheses of non-radioactive standards 1–4

All reference compounds were synthesized in-house based on previously reported methods.[19,20] Compounds were identified with HRMS, ^{1}H NMR and ^{13}C NMR spectroscopy. Compound purities were determined with analytical HPLC and exceeded 98%.

Syntheses of iodo precursors 16–19

General Procedure A: *alkylation of indoles.* A mixture of the appropriate indole (1.0 equiv.), powdered KOH (2.2 equiv.), and tetrabutylammonium bromide (10 mol%), was stirred in neat epichlorohydrin (18 equiv.) at room temperature for 1 h before being directly subjected to flash column chromatography.

General Procedure B: *ring-opening of epoxides*. A mixture of the appropriate epoxide (1.0 equiv.), the appropriate substituted phenol (1.0 equiv.), and 4-dimethylaminopyridine (20 mol%) was dissolved in a small volume of DCM before being concentrated *in vacuo*. The resulting mixture was heated at 120 °C for 1 h before being directly subjected to flash column chromatography.

General Procedure C: *oxidation of secondary alcohols*. To a mixture of the appropriate alcohol (1.0 equiv.) in dry DCM was added Dess-Martin periodinane (1.5 equiv.). The reaction mixture was stirred at room temperature for 4 h, followed by the addition of sodium thiosulfate in saturated $NaHCO_3$ solution. After stirring for 10 min, the reaction mixture was extracted with DCM. The combined organics were dried ($MgSO_4$) and concentrated *in vacuo*. The product was purified with flash column chromatography.

1-(5-Iodo-1H-indol-3-yl)ethan-1-one (**5**): To a solution of 5-iodoindole (4.00 g, 15.6 mmol) and $AlCl_3$ (3.73 g, 28.1 mmol) in DCM (60 mL) was added acetyl chloride (2.31 mL, 28.1 mmol) and the solution stirred at room temperature for 8 h. The reaction mixture was quenched by addition of water and extracted with ethyl acetate. The combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. The product was purified by recrystallization from ethyl acetate to afford **5** (3.80 g, 13.3 mmol, 85%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 12.14−11.98 (bs, 1H), 8.52 (dd, *J* 0.5, 1.6, 1H), 8.32 (s, 1H), 7.49 (dd, *J* 8.52, 1.6, 1H), 7.33 (dd, *J* 8.5, 0.5, 1H), 2.45 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 193.1, 136.2, 135.5, 131.2, 130.1, 128.2, 116.3, 115.0, 86.6, 27.6. m/z (ES^+) $[M+H]^+$ = 284.9 (100%).

1-(5-Iodo-1H-indol-3-yl)-2-methylpropan-1-one (**6**): To a solution of 5-iodoindole (2.83 g, 11.0 mmol) and $AlCl_3$ (2.62 g, 19.8 mmol) in DCM (60 mL) was added isobutyryl chloride (2.08 mL, 19.8 mmol) and the solution stirred at room temperature for 8 h. The reaction mixture was quenched by addition of water and extracted with ethyl acetate. The combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. The product was purified by recrystallization from ethyl acetate to afford **6** (1.46 g, 13.3 mmol, 42%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 12.14−12.00 (bs, 1H), 8.56 (d, *J* 1.7, 1H), 8.32 (d, *J* 3.14, 1H), 7.48 (dd, *J* 8.5, 1.7, 1H), 7.32 (dd, *J* 8.5, 0.5, 1H) 3.50−3.36 (m, 1H), 1.12 (d, *J* 6.7, 6H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 199.9, 136.3, 134.7, 131.2, 130.4, 128.7, 114.9, 114.6, 86.5, 36.3, 20.2. m/z (ES^+) $[M+H]^+$ = 312.9 (100%).

Methyl 5-iodo-1H-indole-3-carboxylate (**7**): To a solution of 5-iodoindole (4.20 g, 17.0 mmol) in dioxane (70 mL) was added pyridine (13.7 mL) and trichloroacetyl chloride (9.5 mL, 85.0 mmol) and the solution stirred at 80 °C for 2.5 h. The cooled reaction mixture was then poured into water and extracted with ethyl acetate. The combined organics were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was dissolved in anhydrous methanol (100 mL) and NaOH (235 mg, 5.88 mmol) was added and the reaction mixture heated at 80 °C for 30 min. The cooled reaction mixture was concentrated *in vacuo* and the partitioned between water and ethyl acetate and extracted with ethyl acetate. The combined organics were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The product was purified by recrystallization from ethyl acetate to afford **7** (3.85 g, 12.7 mmol, 75%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 8.32 (d, 1H), 8.09 (s, 1H), 7.49 (dd, 1H), 7.35 (d, 1H), 3.81 (s, 3H).

5-(5-Iodo-1H-indol-3-yl)-3-methyl-1,2,4-oxadiazole (**8**): To a solution of *N*-hydroxyacetamidine (956 mg, 12.9 mmol) in THF (150 mL) was added NaH (60% dispersion in mineral oil, 511 mg) and the mixture stirred at room temperature for 1 h. After addition of **7** (3.85 g, 12.7 mmol), the mixture was heated under reflux for 24 h. Water and ethyl acetate were added and THF was removed *in vacuo*. The concentrated mixture was extracted with ethyl acetate and the combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. The product was purified by recrystallization from ethyl acetate to afford **8** (2.67 g, 8.17 mmol, 63%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 8.44 (dd, *J* 1.7, 0.5, 1H), 8.35 (s, 1H), 7.56 (dd, *J* 8.5, 1.7, 1H), 7.42 (dd, *J* 8.5, 0.5, 1H), 2.41 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 172.4, 167.3, 136.1, 131.7, 131.5, 129.2, 128.8, 127.2, 115.5, 99.7, 86.3. m/z (ES^+) $[M+H]^+$ = 324.9 (100%).

1-(5-Iodo-1-(oxiran-2-ylmethyl)-1H-indol-3-yl)ethan-1-one (**9**): Prepared according to General Procedure A using **5** (3.56 g, 12.5 mmol), KOH (1.54 mg, 27.5 mmol), tetrabutyl ammonium bromide (402 mg, 1.25 mmol) and epichlorohydrin (18 mL, 225 mmol). Purification by flash column chromatography eluting with EtOAc/pet ether (10:90 to 50:50) afforded **9** (3.7 g, 10.9 mmol, 87%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 8.65 (dd, *J* 0.5, 1.7, 1H), 7.61 (s, 1H), 7.46 (dd, *J* 1.7, 7.6, 1H), 7.05 (d, *J* 8.7, 1H), 4.47 (dd, *J* 5.9, 15.2, 1H), 3.26-3.20 (m, 1H), 2.78 (t, *J* 4.2, 1H), 2.40 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 192.8, 135.4, 132.0, 131.4, 128.3, 116.7, 111.7, 86.9, 50.6, 48.4, 45.0, 27.5. m/z (ES^+) $[M+H]^+$ = 342.3 (100%).

1-(5-Iodo-1-(oxiran-2-ylmethyl)-1H-indol-3-yl)-2-methylpropan-1-one (**10**): Prepared according to General Procedure A using **6** (1.44 g, 4.60 mmol), KOH (567 mg, 10.12 mmol), TBAB (148 mg, 0.460 mmol) and epichlorohydrin (4.2 mL, 82.8 mmol). Then purified by flash column chromatography eluting with EtOAc/pet ether (10:90 to 50:50) afforded **10** (1.61 g, 4.37 mmol, 95%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 8.72 (d, *J* 1.6,

1H), 7.66 (s, 1H), 7.46 (dd, *J* 1.7, 8.6, 1H), 7.06 (d, *J* 8.6, 1H), 4.45 (dd, *J* 2.4, 15.2, 1H), 3.99 (dd, *J* 5.9, 15.2, 1H), 3.26−3.19 (m, 1H), 2.76 (t, *J* 4.2, 1H), 2.37 (dd, *J* 2.5, 4.6, 1H), 1.15 (d, *J* 6.7, 6H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 199.0, 135.3, 133.6, 130.9, 130.7, 127.8, 114.1, 110.6, 85.9, 49.3, 47.4, 43.9, 36.2, 18.7. m/z (ES$^+$) $[M+H]^+$ = 370.3 (100%).

5-(5-Iodo-1-(oxiran-2-ylmethyl)-1H-indol-3-yl)-3-methyl-1, 2, 4-oxadiazole (**11**): Prepared according to General Procedure A using **8** (2.60 g, 7.95 mmol), KOH (978 mg, 17.4 mmol), tetrabutyl ammonium bromide (260 mg, 0.807 mmol) and epichlorohydrin (11 mL, 140 mmol). Then purified by flash column chromatography eluting with EtOAc/pet ether (10:90 to 40:60) followed by recrystallization from ethyl acetate afforded **11** (1.40 g, 3.67 mmol, 46%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 8.45−8.43 (m, 1H), 8.38 (s, 1H), 7.64−7.59 (m, 2H), 4.70 (dd, *J* 3.4, 15.2, 1H), 4.35 (dd, *J* 6.5, 15.2, 1H), 3.42-3.35 (m, 1H) 2.80 (dd, *J* 4.1, 4.8, 1H), 2.58 (dd, *J* 2.6, 4.8 1H), 2.41 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 172.0, 167.4, 135.6, 135.4, 134.6, 131.7, 128.9, 127.4, 114.6, 99.2, 86.9, 50.7, 48.7, 44.0 11.7. m/z (ES$^+$) $[M+H]^+$ = 382.3 (100%).

1-(1-(2-Hydroxy-3-(4-phenoxyphenoxy)propyl)-5-iodo-1H-indol-3-yl)-2-methylpropan-1-one (**12**): Prepared according to General Procedure B using **10** (1.90 g, 5.15 mmol), 4-phenoxyphenol (958 mg, 5.15 mmol), and 4-dimethylaminopyridine (121 mg, 0.992 mmol). Purification by flash column chromatography eluting with EtOAc/pet ether (20:80 to 30:70) afforded **12** (1.46 g, 2.63 mmol, 51%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 8.45 (s, 1H), 8.32 (s, 1H), 7.51−7.48 (m, 2H), 7.39−7.28 (m, 2H), 7.39−7.28 (m, 3H), 7.10−7.05 (m, 1H), 6.95−6.86 (m, 4H), 6.81−6.76 (m, 1H), 5.50 (d, *J* 5.2, 1H), 4.48 (dd, 3.7, *J* 14.1, 1H), 4.36-4.20 (m, 2H), 3.96−3.89 (m, 2H), 3.40−3.32 (m, 1H), 6.37 (dd, *J* 1.7, 6.8, 6H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 199.5, 158.4, 155.1, 150.1, 138.4, 136.9, 131.1, 130.4, 130.3, 130.2, 129.1, 129.0, 123.1, 121.4, 121.1, 117.8, 117.3, 116.3, 114.0, 113.5, 86.9, 70.3, 68.1, 50.0, 36.6, 20.2, 20.1. m/z (ES$^+$) $[M+H]^+$ = 556.5 (100%).

1-(1-(2-Hydroxy-3-(4-(4-(trifluoromethyl)phenoxy)phenoxy)propyl)-5-iodo-1H-indol-3-yl)ethan-1-one (**13**): Prepared according to General Procedure B using **9** (3.53 g, 10.3 mmol), 4-(4-trifluoromethylphenoxy)phenol (2.62 g, 10.3 mmol), and 4-dimethylaminopyridine (253 mg, 2.07 mmol). Purification by flash column chromatography eluting with EtOAc/pet ether (20:80 to 50:50) afforded **13** (4.71 g, 7.92 mmol, 77%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 8.56−8.53 (m, 1H), 8.32 (s, 1H), 7.74−7.78 (m, 2H), 7.14−7.01 (m, 6H), 5.34 (d, *J* 5.2, 1H), 4.48 (dd, *J* 3.5, 13.9, 1H), 4.37-4.17 (m, 2H), 4.00-3.92 (m, 2H), 2.41 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 192.7, 161.8, 156.0, 148.6, 139.2, 136.8, 131.1, 130.2, 128.6, 127.8, (q, *J* 3.6), 122.1, 117.4, 116.5, 115.3, 114.0, 89.9, 70.5, 68.2, 50.0, 27.6. m/z (ES$^+$) $[M+H]^+$ = 596.4 (100%).

1-(5-Iodo-3-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-indol-1-yl)-3-(4-phenoxyphenoxy)propan-2-ol (**14**): Prepared according to General Procedure B using **11** (600 mg, 1.57 mmol), 4-phenoxyphenol (293 mg, 1.58 mmol), and 4-dimethylaminopyridine (37 mg, 0.303 mmol). Purification by flash column chromatography eluting with EtOAc/pet ether (30:70) afforded **14** (650 mg, 1.15 mmol, 73%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 8.48−8.42 (m, 1H), 8.34 (s, 1H), 7.58 (d, *J* 1.2, 2H), 7.36−7.32 (m, 2H), 7.08 (dt, *J* 1.2, 7.46, 1H), 6.95−6.89 (m, 2H), 5.53 (d, *J* 5.3, 1H), 4.54 (dd, *J* 3.6, 14.2, 1H), 4.38 (dd, *J* 7.5, 14.2, 1H), 4.27−4.16 (m, 1H), 3.93 (dd, *J* 2.2, 5.3, 1H), 2.41 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 172.1, 167.3, 158.4, 155.1,150.1, 136.6, 135.2, 131.4, 130.4, 128.9, 127.5, 123.1, 121.1, 117.8, 116.3, 114.6, 98.9, 86.7, 70.4, 68.3, 50.1, 11.7. m/z (ES$^+$) $[M+H]^+$ = 568.3 (100%).

1-(5-Iodo-3-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-indol-1-yl)-3-(4-octylphenoxy)propan-2-ol (**15**): Prepared according to General Procedure B using **11** (690 mg, 1.81 mmol), 4-octylphenol (373 mg, 1.81 mmol), and 4-dimethylaminopyridine (94 mg, 0.770 mmol). Purification by flash column chromatography eluting with EtOAc/pet ether (20:80 to 40:60) afforded **15** (285 mg, 0.486 mmol, 27%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 8.43 (t, *J* 1.2, 1H), 8.33 (s, 1H), 7.56 (s, 2H), 7.09 (d *J* 8.6, 2H), 6.85 (d, *J* 8.6, 2H), 5.49 (d, *J* 5.6, 1H), 4.54 (dd *J* 3.6, 14.3, 1H), 4.36 (dd, *J* 7.6, 14.3, 1H), 4.24−4.13 (m, 1H), 3.94−3.84 (m 2H), 2.41 (s, 3H), 1.58-1.45 (m, 2H), 1.32−1.18 (m, 12H), 0.89−0.81 (m, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 172.2, 167.3, 156.9, 136.6, 135.2, 135.0, 131.4, 129.6, 128.9, 127.4, 114.8, 114.5, 98.8, 86.6, 70.0, 68.3, 50.1, 34.7, 31.7, 31.6, 29.3, 29.1, 29.0,22.5, 14.4, 11.7. m/z (ES$^+$) $[M+H]^+$ = 588.5 (100%).

1-(5-Iodo-1-(2-oxo-3-(4-phenoxyphenoxy)propyl)-1H-indol-3-yl)-2-methylpropan-1-one (**16**): Prepared according to General Procedure C using **12** (1.46 g, 2.63 mmol), Dess-Martin periodinane reagent (1.66 g, 3.92 mmol) in DCM (30 mL). Purification by flash column chromatography eluting with EtOAc/pet ether (10:90 to 30:70) afforded **16** (1.39 g, 2.5 mmol, 96%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 8.84 (d, 1H), 7.67 (1H, s), 7.55 (dd, 1H), 7.31−7.38 (m, 2H), 6.87−7.14 (m, 8H), 5.27 (s, 2H), 4.69 (s, 2H), 3.26 (sept, 1H), 1.27 (d, 6H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 200.9, 199.6, 158.3, 154.4, 150.6, 138.5, 137.2, 131.4, 130.4, 128.8, 123.2, 121.0, 117.9, 116.5, 114.0, 113.9, 87.1, 71.8, 53.3, 20.1. HRMS (ESI-TOF) calcd for $C_{27}H_{25}NIO_4$ $[M+H]^+$ 554.0815, found 554.0823.

1-(3-acetyl-5-iodo-1H-indol-1-yl)-3-(4-(4-(trifluoromethyl)phenoxy)phenoxy)propan-2-one (**17**): Prepared according to General Procedure C using **13** (4.64 g, 7.80 mmol), Dess-Martin periodinane reagent (4.92 g, 11.6 mmol) in DCM (100 mL). Purification by flash column chromatography eluting with EtOAc/pet ether (40:60 to 60:40) afforded **17** (2.4 g, 4.05 mmol, 52%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 8.78 (s, 1H), 7.64 (s, 1H), 7.53−7.59 (m, 3H), 6.93−7.10 (m, 6H), 6.89 (d, 1H), 5.27 (s, 2H), 4.71 (s, 2H), 2.51 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz,) δ_C 200.8, 192.8, 161.7, 155.3, 149.0, 139.2, 137.1, 131.4, 130.2, 128.3, 127.9, 127.8, 126.6, 123.5, 123.0, 122.1, 117.5, 116.7, 115.9, 113.9, 87.2, 53.3, 27.7. HRMS (ESI-TOF) calcd for $C_{26}H_{19}F_3INO_4$ $[M+H]^+$ = 594.0380, found 594.0384.

1-(5-Iodo-3-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-indol-1-yl)-3-(4-phenoxyphenoxy)propan-2-one (**18**): Prepared according to General Procedure C using **14** (590 mg, 1.04 mmol), Dess-Martin periodinane reagent (656 mg, 1.54 mmol) in DCM (3 mL). Recrystallization from ethyl acetate afforded **18** (450 mg, 0.796 mmol, 77%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 8.68 (d, 1H), 7.83 (s, 1H), 7.60 (dd, 1H), 7.31−7.38 (m, 2H), 7.10 (tt, 1H), 6.91−7.06 (m, 7H), 5.31 (s, 2H,), 4.71 (s, 2H), 2.49 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 200.8, 172.0, 167.4, 158.3, 154.4, 150.6, 137.0, 135.3, 131.7, 130.4, 128.9, 127.9, 123.2, 121.0, 117.9, 116.5, 114.4, 99.5, 86.9, 71.7, 53.5, 11.7. HRMS (ESI-TOF) calcd for $C_{26}H_{20}IN_3O_4$ $[M+H]^+$ 566.0565, found 566.0571.

1-(5-Iodo-3-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-indol-1-yl)-3-(4-octylphenoxy)propan-2-one (**19**): Prepared according to General Procedure C using **15** (274 mg, 0.467 mmol), Dess-Martin periodinane reagent (294 mg, 0.695 mmol) in DCM (10 mL). Recrystallization from ethyl acetate afforded **19** (110 mg, 0.162 mmol, 35%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 8.66 (d, 1H), 7.57 (dd, 1H), 7.18 (d, 2H), 6.85−6.90 (m, 3H), 5.29 (s, 2H), 4.71 (s, 2H), 2.59 (t, 2H), 2.48 (s, 3H), 1.57−1.66 (m, 2H), 1.27−1.36 (m, 10H), 0.89 (t, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 200.9, 172.0, 167.4, 156.1, 136.9, 135.6, 135.3, 131.7, 129.7, 128.9, 127.3, 114.9, 114.4, 99.4, 86.9, 53.5, 34.7, 31.7, 31.6, 29.3, 29.1, 29.0, 22.5, 14.4, 11.7. HRMS (ESI-TOF) calcd for $C_{28}H_{32}IN_3O_3$ $[M+H]^+$ 586.1555, found 586.1561.

Radiochemistry

No-carrier-added [^{11}C]carbon dioxide (~89 GBq) was prepared with the ^{14}N(p,α)^{11}C nuclear reaction by bombarding a nitrogen-1% oxygen gas target (initial pressure 300 psi) with a proton beam (16 MeV, 45 μA) from a cyclotron (PETrace, GE) for 40 min. Radiochemistry was performed on a modified Synthia platform controlled by in-house developed software based on Labview.[48,49,56] [^{11}C]Carbon dioxide was first collected in a stainless steel trap filled with molecular sieves (13X; Grace, Chicago, IL) at room temperature. The trap was purged with helium at 80 mL/min for 60 s to remove oxygen. [^{11}C]Carbon dioxide was released in a helium stream (16 mL/min) at ~ 270 °C and concentrated in a liquid nitrogen cryogenic trap filled with silica gel. [^{11}C]Carbon dioxide was then released from the

first cryo-trap in helium (10 mL/min) by warming the trap with a halogen lamp and then passed over molybdenum wire (99.97%, 0.05 mm diameter, Strem Chemicals, Newburyport, MA) in a quartz tube (22 cm length, 0.7 cm i.d.) heated at 875 °C to produce [^{11}C]carbon monoxide. [^{11}C]Carbon monoxide was first concentrated cryogenically on silica gel in a second stainless steel trap cooled with liquid nitrogen and then released in helium into the autoclave by warming the trap with a halogen lamp.

[^{11}C]**1**–**4** were each prepared in similar manner. The following is a typical procedure. Iodo precursor **16–19** (1.5–2.0 mg, 2.5–3.6 μmol) and $Pd(PPh_3)_4$ (0.8–1.2 μmol) in THF (80 μL) were loaded into a reagent loop. The mixture was pumped into the autoclave and allowed to react with [^{11}C]carbon monoxide at 180 °C (130 °C for [^{11}C]**4**) for 5 min. The radioactive reaction mixture was collected in a vented glass V-vial (5 mL), and quenched with H_2O (3 mL). ^{11}C-Labeled product was purified by semi-preparative HPLC, equiped with a Luna C18 column (10 μm, 10 × 250 mm, Phenomenex, Torrance, CA). Absorbance was monitored at 254 nm (System Gold 166, Beckman Coulter Inc., Pasadena, CA) while radioactivity was monitored with a pindiode detector (Bioscan Inc., Washington DC). Semi-prep HPLC method (I) was eluted at 6 mL/min with a linear gradient of 5 mM aqueous NH_4OH (A)–MeCN (B), starting with 10% B for 1 min, increasing to 55% B in 14 min, held for 3 min, then increased to 85% B in 2 min and held at 85% B until the end of run. Semi-prep HPLC method (II) was eluted at 6 mL/min with a linear gradient of 5 mM aqueous NH_4OH (A)–MeCN (B), starting with 10% B for 1 min, increasing to 60% B in 14 min, held for 3 min, then increased to 85% B in 2 min and held at 85% B until the end of run. The radioligand fraction was collected and concentrated in the presence of ascorbic acid (0.15 mg) under vacuum at 80 °C as soon as the fraction collection began, and for a further 1 min after fraction collection ended. The radioactive residue was reconstituted in ethanol/saline solution (1:9, 10 mL), ascorbic acid (0.15 mg) and TWEEN (12 mg) to obtain dose for injection (up to 1.5 GBq). When required the formulation was sterilized by filtration through a 0.22 μm sterile filter (Millipore-MP, Waters Corp., Milford, MA). An aliquot (50 or 100 μL) of the formulated product was analysed with radio-HPLC on a Gemini-NX column (5 μm, 4.6 × 250 mm; Phenomenex), eluted at 3 mL/min with a linear gradient of NH_4OH (A, 2.5 mM)–MeCN(B) from 10 to 55% B over 12 min, to obtain radiochemical purity, chemical purity and molar activity. Retention times (t_R) for [^{11}C]**1**–**4** are 5.6, 5.7, 5.2 and 7.9 min, respectively. The identity of ^{11}C-labeled radioligands was verified by co-injection of reference standards on analytical HPLC and by LC-MS analysis of the carrier.

Lipophilicity (log$D_{7.4}$) measurements and stability in aqueous buffer

The value for the distribution coefficient (log$D_{7.4}$) of [^{11}C]**1**–**4** between 1-octanol and sodium phosphate buffer (0.15 M, pH 7.4) was determined with a technique described previously,[57] but with correction for radioligand instability in the buffer phase. The radioligand in ethanol/saline was placed in 0.15 M sodium phosphate buffer for the duration of the study followed by the determination of its radiochemical purity to obtain information on its stability in buffer. The radiochemical composition of the aqueous buffer remaining after their extraction with 1-octanol was determined using high-performance liquid chromatography (HPLC) on a X-Terra C_{18} column (10 μm, 7.8 × 300 mm, Waters Corp.) and a mobile phase of MeOH:H_2O:Et_3N (92.5:7.5:0.1, v/v) at 4.0 mL/min. The HPLC system consisted of Beckman Gold (Beckman Coulter, Inc.) analytic pumps equipped with an in-line photodiode-array detector and a flow-through NaI scintillation detector-rate meter (Bioscan). Data from the radioanalysis were collected and stored with Bio-Chrome Lite software (Bioscan) and analyzed after decay correction of the radiochromatograms.

Tissue stability

The stability of [^{11}C]**1**–**4** was evaluated in human plasma, and in brain homogenates of rat, monkey and human. These tissues had been stored at −70 °C but were thawed on the day of analysis. [^{11}C]**1**–**4** (~370 kBq/10.0 μL) was added to thawed tissues (500 μL), mixed well and incubated at room temperature for 30 min. The stability of [^{11}C]**1** and it distribution into cellular blood elements was determined in freshly obtained anticoagulated (heparin) blood. The stability of [^{11}C]**1**–**4** was obtained by dividing the tissue radiochromatographic composition by the radiochemical purity of the radioligand.

Plasma free fraction

The plasma free fraction (f_p) of [^{11}C]**1**–**4** was measured by ultrafiltration through membrane filters (Centrifree; Millipore), as previously described.[58] Briefly, 740 kBq (~ 4.4 μL) of [^{11}C]**1**–**4** was added to 700 μL of pooled human plasma. The mixture was incubated at room temperature for 10 min. The ultrafiltration components that contained high radioactivity were allowed time until radioactivity decayed to within the optimal range of the γ-counter before they were counted. Quantification of the ultrafiltrates was carried out gravimetrically.

PET imaging

PET imaging experiments were performed in accordance with the Guide for Care and Use of Laboratory Animals[59] and were approved by the National Institute of Mental Health Animal Care and Use Committee. All mice were anesthetized with 1.5% isoflurane. Scans were acquired using microPET Focus 120 (Siemens Medical Solutions, Knoxville, TN) for a duration of 100 min. [^{11}C]**1–4** was individually injected (~ 7.4 MBq) intravenously (i.v.) through a tail vein catheter. Blocking agent (2 mg/kg) was administered i.v. at 30 min before radioligand injection also via tail vein catheter. Images were reconstructed using Fourier rebinning followed by 2D OSEM without attenuation correction. No scatter correction was applied.

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