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Synthesis and evaluation of [¹¹C]PyrATP-1, a novel radiotracer for PET imaging of glycogen synthase kinase-3 β (GSK-3 β)

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Abstract

Introduction—The dysfunction of glycogen synthase kinase-3 β (GSK-3 β) has been implicated in a number of diseases, including Alzheimer's disease. The ability to non-invasively quantify GSK-3 β activity *in vivo* is therefore of critical importance, and this work is focused upon development of inhibitors of GSK-3 β radiolabeled with carbon-11 to examine quantification of the enzyme using positron emission tomography (PET) imaging.

Methods—[¹¹C]PyrATP-1 was prepared from the corresponding desmethyl-piperazine precursor in an automated synthesis module. *In vivo* rodent and primate imaging studies were conducted on a Concorde MicroPET P4 scanner to evaluate imaging properties, and *in vitro* autoradiography studies with rat brain samples were carried out to examine specific binding.

Results—2035 $\pm$ 518 MBq (55 $\pm$ 14 mCi) of [¹¹C]PyrATP-1 were obtained (1–2% noncorrected radiochemical yield at end-of-synthesis based upon [¹¹C]CO₂) with high chemical (>95%) and radiochemical (>99%) purities, and good specific activities (143 $\pm$ 52 GBq/ μ mol (3874 $\pm$ 1424 Ci/mmol)), n = 5. *In vivo* microPET imaging studies revealed poor brain uptake in rodents and non-human primates. Pretreatment of rodents with cyclosporin A resulted in moderately increased brain uptake suggesting Pgp transporter involvement. Autoradiography demonstrated high levels of specific binding in areas of the rodent brain known to be rich in GSK-3 β .

Conclusion—[¹¹C]PyrATP-1 is readily synthesized using standard carbon-11 radiochemistry. However, the poor brain uptake in rodents and non-human primates indicates that the radiotracer is not suitable for the purposes of quantifying GSK-3 β in neurological and psychiatric disorders.

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1. Introduction

Glycogen synthase kinase-3 β (GSK-3 β) is a proline-directed serine/threonine kinase. Dysregulation of GSK-3 β has been implicated in cancer [1], diabetes [2], nociception [3, 4], a range of psychiatric disorders (e.g. bipolar disorder, depression, schizophrenia [5]), and Alzheimer's disease (leading to the GSK-3 hypothesis of Alzheimer's disease [6]). Thus, dysfunction of GSK-3 β could be a prodromal marker for each of these conditions, and GSK-3 β inhibitors are being explored for therapeutic development across this spectrum of diseases and disorders [5, 7–9]. Reflecting this, the ability to non-invasively quantify GSK-3 β *in vivo* is of critical importance. Radiotracers enabling functional positron emission tomography (PET) imaging of this kinase could be used for early detection of GSK-3 β dysregulation, enabling pre-symptomatic diagnosis of the above conditions, and as companion diagnostics to support development of small molecule inhibitors of GSK-3 β for treatment purposes. Despite this, prior work aimed at PET imaging of GSK-3 β has been limited [10–14]. Vasdev and co-workers prepared [¹¹C]AR-A014418 (**1**), but unfortunately the radiotracer did not cross the blood-brain barrier (BBB) despite a log *P* of 2.44 and previously reported central nervous system (CNS) effects [11, 12]. Recently, two syntheses of [¹¹C]SB-216763 (**2**) have been reported [13, 14], and Aigbirhio and co-workers reported preliminary evidence for BBB permeability [14]. For that radiotracer, no further studies of imaging properties or brain pharmacokinetics have been reported.

We are particularly interested in imaging GSK-3 β in the CNS because it is the main enzyme responsible for phosphorylation of tau. Dysfunction of this process is thought to generate the hyperphosphorylated tau that aggregates into tau neurofibrillary tangles in Alzheimer's disease [6, 15], and is aligned with our program developing radiotracers for PET imaging of tau [16]. In our search for a radiotracer that would enable PET imaging of GSK-3 β , we were particularly attracted by a recent report of a family of pyrazines (e.g. PyrATP-1 (**3**)) by Berg and co-workers [17], representing a third structural scaffold for GSK-3 β inhibitors (Figure 1). The lead compound has high affinity for the ATP binding site of GSK-3 β (K_i = 4.9 nM), and >100-fold selectivity for GSK-3 β over cyclin-dependent kinase 2 (CDK2), a related proline-directed serine/threonine kinase with high sequence similarity between ATP binding sites. The reported analogs offer a particularly rich selection of high affinity substrate competitive inhibitors of GSK-3 β that can be readily labeled with carbon-11 or fluorine-18 and, based upon an *in vitro* bovine endothelial cell assay, are predicted to cross the BBB and enter the CNS. This paper reports the synthesis and pre-clinical evaluation of the first example of a radiolabeled inhibitor of GSK-3 β based upon the pyrazine scaffold.

2. Materials and Methods

2.1 Chemistry

2.1.1 General Considerations—All the chemicals employed in the syntheses were sourced commercially from reliable suppliers of fine chemicals, and used without further purification unless otherwise stated. ¹H-Nuclear magnetic resonance (NMR) spectra were obtained at 400 MHz on Varian NMR spectrometer in CD₃OD or CDCl₃ solutions at room temperature with tetramethylsilane (TMS, δ = 0) as an internal standard. ¹³C-NMR spectra we obtained at 100 MHz. Chemical shifts (δ) are reported in ppm and coupling constants are

reported in Hertz. Multiplicity is defined by s (singlet), d (doublet), t (triplet), and m (multiplet). High performance liquid chromatography (HPLC) was performed using a Shimadzu LC-2010A HT system equipped with a Bioscan B-FC-1000 radiation detector. Mass spectra were performed on a VG (Micromass) 70-250-S Magnetic sector mass spectrometer or Micromass AutoSpec Ultima Magnetic sector mass spectrometer employing the electrospray ionization (ESI) method.

2.1.2 3-Amino-6-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)-*N*-(pyridin-3-yl)pyrazine-2-carboxamide (Pyr-ATP-1 Reference Standard 3)—PyrATP-1

reference standard was prepared according to previous reports [17–19], yielding the title compound as a yellow solid (43.8 mg, 8.5% over 3-steps). ¹H-NMR (400 MHz, CDCl₃): δ 9.85 (s, 1H), 8.85 (s, 1H), 8.74 (s, 1H), 8.44 (d, *J* = 4.2 Hz, 1H), 8.30 (d, *J* = 8.3 Hz, 1H), 8.04 (d, *J* = 8.4 Hz, 2H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.37 (dd, *J* = 8.3, 4.8 Hz, 1H), 3.12 (s, 4H), 2.55 (s, 4H), 2.30 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃): δ 154.75, 145.84, 145.69, 141.68, 138.65, 135.20, 134.19, 128.76, 127.27, 126.29, 124.59, 123.95, 54.10, 46.02, 45.75, 29.85. HRMS (ESI) calculated for C₂₁H₂₄N₇O₃S [*M*+H]⁺ 454.1656, found 454.1653. All spectral data were in agreement with literature values [17].

2.1.3 *tert*-Butyl 4-((4-bromophenyl)sulfonyl)piperazine-1-carboxylate (**6**)—1-

Boc-piperazine (3.1605 g, 16.969 mmol, Aldrich) and 4-bromobenzene (2.7037 g, 10.0582 mmol, Aldrich) were stirred in benzene (60 mL) for 4 h at room temperature. The benzene was removed under vacuum. The product was dissolved in CH₂Cl₂ then washed with water followed by 1M NaOH solution and then dried with Na₂SO₄, and concentrated. The crude product was purified by flash chromatography using a silica gel column with 0 to 50% EtOAc in hexane to give **6** a white crystalline solid (2.094 g, 51%), mp = 141.0 – 142.0 °C. ¹H-NMR (400 MHz, CDCl₃): δ 7.72 – 7.66 (m, 2H), 7.65 – 7.57 (m, 2H), 3.58 – 3.44 (m, 4H), 3.06 – 2.91 (m, 4H), 1.41 (s, 9H). ¹³C-NMR (100 MHz, CDCl₃): δ 154.05, 134.52, 132.50, 129.18, 128.31, 128.20, 80.47, 45.82, 28.28. HRMS (ESI) calculated for C₁₅H₂₁BrN₂NaO₄S [*M*+Na]⁺ 427.0298, found 427.0298.

2.1.4 4-((4-((*tert*-Butoxycarbonyl)piperazin-1-yl)sulfonyl)phenyl)boronic acid (**7**)—In a flame dried round-bottomed flask, compound **6** (711.7 mg, 1.756 mmol) and

triisopropyl borate (2.5 mL, 10.83 mmol, Aldrich) in dry THF (30 mL) were cooled to –78 °C under Ar. To this mixture was added a solution of *n*BuLi in THF (6.5 mL of 1.6 M, 10.4 mmol) dropwise over 30 min. The reaction was kept at –78 °C for 3 h, and then overnight at room temperature. The borate was hydrolyzed with water (10 mL) for 30 min then the organic solvents were removed under vacuum. The crude product was dissolved in CH₂Cl₂ and washed with water, dried with Na₂SO₄, and concentrated. Purification by flash chromatography using a silica gel column with 0 to 40% MeOH in CH₂Cl₂ gave **7** as a white powder (227.6 mg, 35%), mp = 115 °C (decomp.). ¹H-NMR (400 MHz, CD₃OD): δ 7.87 (s, 2H), 7.73 (d, *J* = 7.9 Hz, 2H), 3.47 (s, 4H), 3.33 – 3.27 (m, 2H), 2.98 – 2.88 (m, 4H), 1.39 (s, 9H). ¹³C-NMR (100 MHz, CD₃OD): δ 155.87, 135.41, 127.74, 111.40, 81.69, 47.15, 28.49. HRMS (ESI) calculated for C₁₅H₂₃BN₂O₆S [*M*+Na]⁺ 393.1262, found 393.1263.

2.1.5 tert-butyl 4-((4-(5-amino-6-(pyridin-3-ylcarbamoyl)pyrazin-2-yl)phenyl)sulfonyl) piperazine-1-carboxylate (9)—In a round-bottomed flask toluene (10 mL) and EtOH (1 mL) were sparged with Ar for 20 min. To this solvent was added **7** (95.1 mg, 0.257 mmol), **8** (76.3 mg, 0.259 mmol), Pd(dppf)Cl₂ (25.7 mg, 0.0351 mmol, Aldrich), and Na₂CO₃ (1 mL, 2 M), and the reaction was heated overnight at 80 °C under Ar. The reaction solvent was removed under vacuum and the solid dissolved in CH₂Cl₂ followed by washing with water and drying with Na₂SO₄. The crude product was purified by flash chromatography using a silica gel column with 0 to 10% MeOH in CH₂Cl₂ to give compound **9** a yellow crystalline solid (53.8 mg, 39%), mp = 211.0 – 212.5 °C. ¹H-NMR (400 MHz, CDCl₃): δ 9.86 (s, 1H), 8.85 (s, 1H), 8.76 (s, 1H), 8.43 (d, *J* = 4.0 Hz, 1H), 8.29 (d, *J* = 8.4 Hz, 1H), 8.07 (d, *J* = 8.3 Hz, 2H), 7.88 (d, *J* = 8.3 Hz, 2H), 7.37 (dd, *J* = 8.2, 4.7 Hz, 1H), 3.56 – 3.51 (m, 4H), 3.48 (s, 9H), 3.04 (s, 4H). ¹³C-NMR (100 MHz, CDCl₃): δ 164.26, 154.76, 154.23, 145.80, 145.65, 141.69, 140.42, 138.35, 135.32, 134.14, 128.62, 128.23, 127.26, 126.30, 124.55, 123.89, 80.61, 50.85, 46.02, 28.39. HRMS (ESI) calculated for C₂₀H₂₁N₇O₃S [*M*+H]⁺ 440.1499, found 440.1494.

2.1.6 3-Amino-6-(4-(piperazin-1-ylsulfonyl)phenyl)-N-(pyridin-3-yl)pyrazine-2-carboxamide (PyrATP-1 precursor (10))—Protected precursor **9** (21.7 mg, 0.0421 mmol) was dissolved in CH₂Cl₂ (10 mL) and trifluoroacetic acid (TFA) (0.3 mL, 3.89 mmol) and then stirred for 8 h, while monitoring by TLC. The reaction was concentrated and washed with diethyl ether to remove any remaining TFA. The product was then dissolved in CH₂Cl₂ and washed with water and saturated Na₂CO₃ and concentrated to give **10** as a yellow powder in quantitative yield (18.48 mg, 100%), mp = 198 °C (decomp.). ¹H-NMR (400 MHz, CD₃OD): δ 9.56 (s, 1H), 8.93 (s, 1H), 8.89 (d, *J* = 8.7 Hz, 1H), 8.59 (d, *J* = 5.0 Hz, 1H), 8.43 (d, *J* = 8.5 Hz, 2H), 8.03 (dd, *J* = 8.5, 5.6 Hz, 1H), 7.97 (d, *J* = 5.7 Hz, 1H), 7.92 (d, *J* = 8.5 Hz, 2H), 3.34 (s, 8H). ¹³C-NMR (100 MHz, CD₃OD): δ 166.90, 156.57, 147.40, 142.30, 139.00, 138.46, 137.01, 136.99, 135.78, 129.51, 127.67, 124.57, 44.33. HRMS (ESI) calculated for C₂₀H₂₁N₇O₃S [*M*+H]⁺ 440.1499, found 440.1494.

2.2 Radiochemistry

2.2.1 General Considerations—Unless otherwise stated, reagents and solvents were commercially available and used without further purification: Sodium Chloride, 0.9% USP and Sterile Water for Injection, USP were purchased from Hospira; ethanol was purchased from American Regent; HPLC grade acetonitrile was purchased from Fisher Scientific. Shimalite-Nickel was purchased from Shimadzu; iodine was purchased from EMD; phosphorus pentoxide was purchased from Fluka; and molecular sieves were purchased from Alltech; sterile filters were obtained from Millipore; sterile product vials were purchased from Hollister-Stier; C18 Sep-Paks and Porapak-Q were purchased from Waters Corporation. Sep-Paks were flushed with 10 mL of ethanol followed by 10 mL of sterile water prior to use.

2.2.2 Synthesis of [¹¹C]PyrATP-1 ([¹¹C]3)—All steps (Scheme 1) were performed using the integrated functions of a TRACERLab FX_{C-Pro} synthesis module. [¹¹C]CO₂ was delivered from the cyclotron (GE PETtrace 6) and converted by gas-phase methods to [¹¹C]methyl triflate ([¹¹C]CH₃OTf) as previously described [20]. The reaction of gaseous

[¹¹C]CH₃OTf with precursor **10** took place in the FX_{C-Pro} reaction vessel. The vessel was charged with 0.5±0.2 mg of precursor in 100 µL of dimethylformamide at ambient temperature. [¹¹C]CH₃OTf was bubbled into the reaction vessel at a fixed flow rate of 15 mL/min for 3 min. The reaction mixture was then diluted with 1 mL of HPLC mobile phase and purified by semi-preparative HPLC (column: Alltech Econosil C18 10µ, 250 × 10 mm; mobile phase: 20 mM NH₄OAc in 30% MeCN, pH 4.6; flow rate: 3 mL/min). The fraction corresponding to [¹¹C]PyrATP-1 (typically eluting out at 15–16 min) was collected and transferred into a dilution flask containing sterile water (50 mL). The resulting solution was transferred through a Waters C18 (50 mg/1 cc) Sep-Pak to collect the desired product. The C18 Sep-Pak was then washed with sterile water (2 mL) to wash unwanted hydrophilic impurities and residual acetonitrile to waste. [¹¹C]PyrATP-1 was eluted into the collection vial with ethanol for injection, USP (0.5 mL) and diluted with 0.9% sodium chloride for injection, USP (4.5 mL). The final formulation (5 mL of 10% v/v ethanol in saline) was then passed through a 0.22 µm millex-GV filter into a sterile vial to provide [¹¹C]PyrATP-1 ([¹¹C]**3**) (2035 ± 518 MBq (55 mCi ± 14 mCi), n = 5). Radiochemical purities (>99%) and specific activities (143 ± 52 GBq / µmol (3874 ± 1424 Ciµmol)) were determined using analytical HPLC (Column: Alltech Econosil C18 10µ, 250 mm × 4.6 mm; mobile phase: 35:65 / MeCN: 20 mM NH₄OAc, pH = 4.5; oven temperature: 25 °C; uv: 254 nm; flow rate: 1.5 mL/min, t_R=5.66 min). Based on approximately 111 GBq (3 Ci) of [¹¹C]CO₂, overall radiochemical yields were 1 – 2% (non-corrected radiochemical yields at end-of-synthesis based upon [¹¹C]CO₂) and were not optimized. pH was determined to be 5.5 using pH-indicator strips, and all doses were confirmed to be clear, colorless and free of particulate matter.

2.3 Pre-clinical Evaluation

2.3.1 General Considerations—All animal studies were performed at the University of Michigan in accordance with the standards set by the University Committee on Use and Care of Animals (UCUCA).

2.3.2 Autoradiography—Rat brains (n = 3) were sectioned coronally at the level of the anterior caudate putamen/nucleus accumbens. Two adjacent 20 µm sections per animal were thaw-mounted onto poly-L-lysine subbed slides, and pre-incubated in PBS-EDTA, pH 7.4 in two cytomailers for 5 min at 25 °C. One slide per animal was then incubated with [¹¹C]PyrATP (56 µCi) for total binding. The second slides were incubated with [¹¹C]PyrATP (56 µCi) in the presence of a 500-fold excess of the unlabeled reference standard to identify non-specific binding. All slides were incubated for 10 min at 25 °C, and then rinsed with 2 × 1 min washes of PBS-EDTA (pH 7.4) at 4 °C, followed by distilled water, before drying and exposing to Fuji phosphorimager plates for 5 min. Plates were read and analyzed using a GE Typhoon FLA 7000 (Figure 2).

2.3.3 Rodent MicroPET Imaging—Rodent imaging studies were done using female Sprague Dawley rats (n = 2, animal weights = 247 – 267 g). The animals were anesthetized (isoflurane), intubated, and positioned in a Concorde MicroPET P4 scanner. Following a transmission scan, the animals were injected i.v. (via tail vein catheter as a bolus over 1 min) with [¹¹C]PyrATP-1 (38.11 MBq (1.03 mCi) in 80 µL of ethanolic saline), and the head

imaged for 60 min (12×5 min frames). Emission data were corrected for attenuation and scatter, and reconstructed using the 3D maximum a priori (3D MAP) method (Figure 3a). Time–radioactivity curves are not reported due to the lack of brain uptake.

2.3.4 Rodent Pgp Blocking Studies—The imaging experiment described in the previous section was repeated using the same female Sprague Dawley rats ($n = 2$, animal weights = 247 – 267 g) pre-treated with cyclosporin A (manufactured for Bedford Labs, Bedford, OH. Lot # 1856175, each mL contains cyclosporin USP 50 mg; polyoxyethyated castor oil of 650 mg; and absolute alcohol 33.2% (v/v)) per the procedure reported by Blanckaert *et al.* [21]. Each rat was injected i.v. with 50 mg/kg (approx. 0.25 mL of stock was diluted to 1.0 mL with isotonic saline) over a 3 minute infusion, one hr. prior to administration of the [^{11}C]PyrATP-1 (25.9 MBq (0.70 mCi) in 60 μL of ethanolic saline). The head was then imaged for 60 min (12×5 min frames). Emission data were corrected for attenuation and scatter, and reconstructed using the 3D MAP method (Figure 3b). By using a summed image, regions of interest (ROI) were drawn over the whole brain on multiple planes, and the volumetric ROIs were then applied to the full dynamic data set to generate time–radioactivity curves (Figure 4).

2.3.5 Primate MicroPET Imaging—Studies were performed in a single young mature female rhesus monkey (6.2 kg). PET imaging was done using the Concorde Microsystems MicroPET P4 tomograph. The animal was anesthetized (isoflurane) and intubated, a venous catheter was inserted into one hindlimb and the animal positioned on the bed of the MicroPET gantry. A head-holder was used to prevent motion artifacts. Isoflurane anesthesia was continued throughout the study. After completion of the transmission scan, the animal was injected with [^{11}C]PyrATP-1 (165.75 MBq (4.48 mCi) in 0.53 mL of ethanolic saline) as a bolus over 1 min. Collection of emission data was started with radiotracer injection and continued for 60 min (5×1 min – 2×2.5 min – 2×5 min – 4×10 min frames). Emission data were corrected for attenuation and scatter, and reconstructed using the 3D MAP method (Figure 3c). Time–radioactivity curves are not reported due to the lack of brain uptake.

2.3.6 Log *P* Determination—PyrATP-1 reference standard **3** (1 mg) was dissolved in a 1:1 mixture of 1-octanol and water (2 mL). The resulting solution was mixed with a vortex mixer for 5 min, and then allowed to settle for 30 min. 3×100 μL samples were carefully removed from each layer and analyzed by HPLC. The mean values of the area under the curve for each group were taken, and used to calculate a log $P_{\text{oct/water}}$ of 1.98.

3. Results and Discussion

3.1 Chemical and Radiochemical Syntheses

The work commenced with the synthesis of the requisite unlabeled reference standard **3**, and the corresponding desmethyl-piperazine precursor **10** (Scheme 1). Synthesis of the reference standard proceeded from commercially available reagents according to literature procedures [17–19], and PyrATP-1 reference standard **3** was obtained in 8.5% yield over 3-steps. All analytical data was in agreement with literature reports.

To synthesize the precursor, 1-Boc-piperazine **4** was reacted with 4-bromobenzenesulfonyl chloride **5**, using Keasling's procedure [18], to generate **6**. Attempts to convert bromide **6** to the corresponding boronic acid **7** using the procedure of Berg *et al.* proved problematic in our hands [17], and so a modified procedure based upon Berg and Hellberg's use of boron tri-isopropoxide [19], and subsequent hydrolysis, was employed to obtain **7**. Suzuki cross-coupling of Boc-protected boronic acid **7** with bromo-pyrazine **8** (prepared from commercially available 3-aminopyridine according to literature procedures [17]) yielded protected precursor **9**. Deprotection of the Boc-group proceeded in quantitative yield upon treatment with trifluoroacetic acid (TFA) to provide desmethyl-precursor **10** in 7% yield over 4-steps. The radiosynthesis of [^{11}C]PyrATP-1 ([^{11}C]**3**) was then achieved by reaction of desmethyl-precursor **10** with high specific activity [^{11}C]MeOTf (Scheme 1). The radiosynthesis was straightforward and provided reasonable yields of [^{11}C]PyrATP-1 (2035 ± 518 MBq (55 mCi ± 14 mCi), 1 – 2% (noncorrected end-of-synthesis yield based upon 111 GBq (3 Ci) of [^{11}C]CO₂), $n = 5$). Radiochemical purities (>99%) and specific activities (143 ± 52 GBq / μmol (3874 ± 1424 Ci/ μmol)) were determined using analytical HPLC.

3.2 In Vitro Autoradiography

Rodent brain sections ($n = 3$) were incubated with [^{11}C]PyrATP-1, washed, dried and then exposed to phosphoimager plates (Figure 2). GSK-3 β is abundant throughout the brain and analysis of the phosphoimager plates supports this, revealing high total binding (blue areas) throughout the cortex, caudate putamen and nucleus accumbens, all areas of the rodent brain known to be rich in GSK-3 β [22–24]. Repeating the experiments with adjacent brain sections of each of the 3 animals in the presence of 500-fold excess of unlabeled PyrATP-1 reference standard identified moderate levels of non-specific binding throughout the entire brain sections. Densitometrical analysis of the images from the phosphorescence imager revealed that 55% of the total signal was attributable to specific binding, and the remaining 45% represented areas of non-displaceable binding.

3.3 In Vivo MicroPET Imaging

The ease of synthesis of [^{11}C]PyrATP-1 and high levels of specific binding in GSK-3 β -rich regions of the rodent brain encouraged us to examine the imaging properties of the new radiotracer. The potential for imaging GSK-3 β with [^{11}C]PyrATP-1 was initially evaluated in normal rats ($n = 2$) using microPET imaging, but there was no uptake of the radiotracer in the rodent brain (Figure 3a). This was a surprising finding given that pyrazine **3** was determined to have a log P of 1.98 in this work, and has previously been evaluated in a bovine endothelial cell assay where it showed good permeability (15×10^{-3} cm/min) and was thus predicted to have good penetration into the CNS [17, 25]. We therefore considered the possibility of BBB efflux transporter involvement such as the P-glycoprotein (Pgp) transporter. To test this, rodent microPET imaging ($n = 2$) was repeated in rodents pre-treated with 50 mg/kg cyclosporin A to block the Pgp 60 minutes prior to i.v. injection of the radiotracer (Figure 3b). Time-radioactivity curves for the whole brain were determined for these scans (Figure 4), and showed a steady increase of radioactivity in the brain with marginal peak uptake of 65,675 Bq/mL (~ 1775 nCi/mL) approximately 40 min post-injection of the radiotracer, corresponding to a peak SUV of 0.65. This is consistent with

trapping of the radiotracer in the brain, and suggests that PyrATP-1 is an irreversible inhibitor of GSK-3 β within the timeframe of a PET scan.

Given that Pgp transporter expression is significantly higher in rodents than primates [26], and that we have previously seen species differences in brain uptake of radiotracers because of this [16], we chose to also evaluate [^{11}C]PyrATP-1 in rhesus macaque ($n = 1$). However in this case, the lack of brain uptake was found to be interspecific, and no brain uptake was apparent from the primate scan either (Figure 3c).

4. Conclusions

[^{11}C]PyrATP-1, a novel radiotracer based upon a literature compound with high affinity and selectivity for GSK-3 β , has been successfully synthesized in acceptable radiochemical yield, high radiochemical purity and good specific activity. However, *in vivo* microPET imaging studies revealed poor brain uptake in rodents and non-human primates indicating that the radiotracer is not suitable for the purposes of quantifying GSK-3 β in neurological and psychiatric disorders. This work emphasizes that high affinity, good selectivity, an appropriate log P value for brain penetration, and *in vitro* evidence of specific binding and BBB permeability do not mean that a compound will make a good radiotracer. *In vitro* models of BBB permeability can fail, demonstrating the value of *in vivo* studies when developing new radiotracers.

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Figure 1.
Scaffolds of GSK-3β inhibitors adapted for PET imaging

Figure 2.
Representative autoradiography images showing high specific binding of [^{11}C]PyrATP-1 throughout the cortex, caudate putamen and nucleus accumbens of the rat brain

Figure 3. Representative microPET images following i.v. injection of $[^{11}\text{C}]\text{PyrATP-1}$ in baseline rodents (a); rodents pre-treated with 5 mg/kg cyclosporin A 60 minutes prior to injection of the radiotracer (b), and baseline non-human primates (c). All images are summed images 0 – 60 minutes following i.v. injection of the radiotracer.

Figure 4.
Time-radioactivity curves for rodent whole brain following pre-treatment with cyclosporin
A

Scheme 1.

Synthesis of desmethyl-piperazine precursor PyrATP-1 (**10**) and radiosynthesis of $[^{11}\text{C}]$ PyrATP-1 ($[^{11}\text{C}]$ **3**)