

Synthesis and Initial *in Vivo* Studies with [¹¹C]SB-216763: The First Radiolabeled Brain Penetrative Inhibitor of GSK-3

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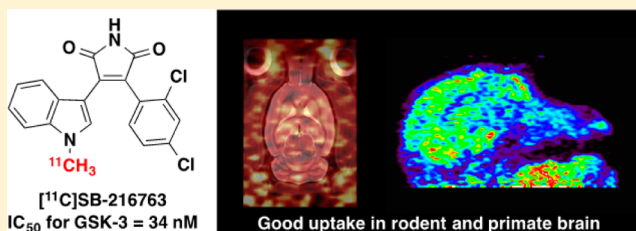
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Supporting Information

ABSTRACT: Quantifying glycogen synthase kinase-3 (GSK-3) activity *in vivo* using positron emission tomography (PET) imaging is of interest because dysregulation of GSK-3 is implicated in numerous diseases and neurological disorders for which GSK-3 inhibitors are being considered as therapeutic strategies. Previous PET radiotracers for GSK-3 have been reported, but none of the published examples cross the blood–brain barrier. Therefore, we have an ongoing interest in developing a brain penetrating radiotracer for GSK-3. To this end, we were interested in synthesis and preclinical evaluation of [¹¹C]SB-216763, a high-affinity inhibitor of GSK-3 ($K_i = 9$ nM; $IC_{50} = 34$ nM). Initial radiosyntheses of [¹¹C]SB-216763 proved ineffective in our hands because of competing [3 + 3] sigmatropic shifts. Therefore, we have developed a novel one-pot two-step synthesis of [¹¹C]SB-216763 from a 2,4-dimethoxybenzyl-protected maleimide precursor, which provided high specific activity [¹¹C]SB-216763 in 1% noncorrected radiochemical yield (based upon [¹¹C]CH₃I) and 97–100% radiochemical purity ($n = 7$). Initial preclinical evaluation in rodent and nonhuman primate PET imaging studies revealed high initial brain uptake (peak rodent SUV = 2.5 @ 3 min postinjection; peak nonhuman primate SUV = 1.9 @ 5 min postinjection) followed by washout. Brain uptake was highest in thalamus, striatum, cortex, and cerebellum, areas known to be rich in GSK-3. These results make the arylindolemaleimide skeleton our lead scaffold for developing a PET radiotracer for quantification of GSK-3 density *in vivo* and ultimately translating it into clinical use.

KEYWORDS: Positron emission tomography, glycogen synthase kinase (GSK-3), carbon-11, neuroimaging, brain PET



Glycogen synthase kinase-3 (GSK-3), which consists of two isoforms (GSK3 α and GSK3 β), has been identified as an enzyme involved in glycogen metabolism, with the highest abundance in the brain.¹ Recent research has shown that GSK-3 β acts as a key component of many cellular and physiological events including cellular growth, apoptosis, and neuropathological events,^{2,3} but its dysregulation is known to play a role in multiple diseases such as diabetes,⁴ neurodegenerative conditions such as Alzheimer's disease,^{5,6} Parkinson disease,⁷ neurological disorders,⁸ pain,^{9,10} and cancer.^{11–13} In light of this, GSK-3 inhibitors are being developed as therapeutics across this disease spectrum.^{2,8,14–22}

Reflecting its role in the pathogenesis of this disease spectrum, there is an important need for tools that enable quantification of GSK-3 *in vivo*. From a diagnostic perspective, early detection of the dysregulation of GSK-3 could prove to be a prodromal biomarker for these conditions enabling early diagnosis and treatment initiation, while the ability to quantify GSK-3/ligand interactions can be used to understand target

engagement by new GSK-3 inhibitors in therapeutic discovery pipelines and, ultimately, aid in developing dosage regimens.

Positron emission tomography (PET) is a widely used *in vivo* imaging technique, which can be applied to quantify enzyme levels and activity in the living brain. The former can be accomplished using a radiolabeled inhibitor of the enzyme, while the latter would require a radiolabeled substrate. However, to date there are no known small molecule substrates for GSK-3. Hence, we are interested in development of a PET radiotracer based upon a GSK-3 inhibitor that will allow us to noninvasively quantify the density of GSK-3 in the living animal brain. A number of GSK-3 inhibitors from various chemical classes have been identified, show varying potencies and isoform selectivity, as reported in recent literature.^{2,8,14–22} However, previous reports of PET radiotracers for GSK-3 are

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limited (Figure 1).^{23–27} The first example was [¹¹C]N-(4-methoxybenzyl)-N'-(5-nitro-1,3-thiazole-2-yl)urea ([¹¹C]AR-

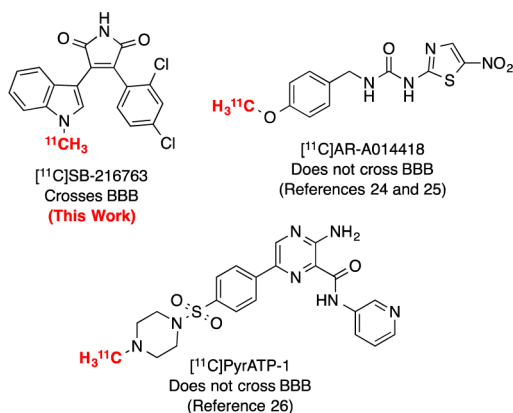


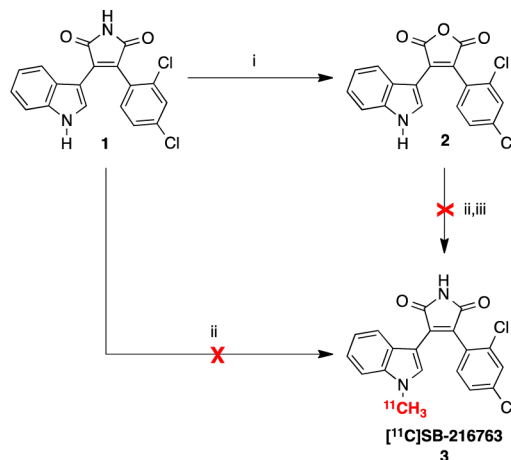
Figure 1. PET Radiotracers for GSK-3.

A014418), reported by Vasdev and colleagues.^{24,25} Although the central nervous system (CNS) effect following peripheral administration of AR-A014418 into rats has been demonstrated,²⁸ the radioactive [¹¹C]AR-A014418 showed no brain penetration, according to *ex vivo* biodistribution studies conducted by the Vasdev group. We recently reported the synthesis and evaluation of [¹¹C]PyrATP-1,²⁶ a GSK-3 β inhibitor based upon the pyrazine scaffold initially reported by Berg and colleagues²¹ and demonstrated specific binding in rat brain *ex vivo* autoradiography experiments. However, despite predictions of good CNS permeability from bovine endothelial cell assays,²¹ [¹¹C]PyrATP-1 also did not enter the brain and is unsuitable for neuroimaging purposes.

In our continuing efforts to develop a brain penetrative GSK-3 radiotracer, we next turned our attention to (3-(2,4-dichlorophenyl)-4-(1-[¹¹C]methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione) (SB-216763), an arylindolemaleimide with high affinity for both isoforms of GSK-3 ($K_i = 9$ nM; $IC_{50} = 34$ nM) that was originally reported by Coghlan and colleagues.²⁹ SB-216763 inhibits the enzyme in an ATP competitive manner, has good selectivity for GSK-3 over other protein kinases, and has been widely used in preclinical studies with promising results to date.³⁰ While a synthesis of [¹¹C]SB-216763 was reported in 2011 by Zheng and co-workers,²⁷ no biological evaluation of the radiotracer has been reported to date. The goal of the current work was therefore evaluation of the imaging properties of [¹¹C]SB-216763, including brain uptake and washout, in rodents and nonhuman primates.

Work commenced with attempts to synthesize [¹¹C]SB-216763 using Zheng's procedure by ¹¹C-methylation of the maleimide anhydride precursor **2**.²⁷ The synthesis of precursor **2** proceeded in 21% yield from **1** when synthesized on gram scale, although no product was obtained when conducted on a smaller scale. Unlabeled SB-216763 reference standard (necessary for confirming identity of the carbon-11 labeled radiotracer) could either be prepared using literature procedures²⁷ or purchased directly (Sigma-Aldrich). Precursor **2** was subjected to Zheng's procedure (Scheme 1): treatment with [¹¹C]CH₃I and subsequent conversion to the maleimide by treatment with lithium hexamethyldisilazane (LiHMDS). Regrettably, this approach yielded no product in either of our laboratories, but rather complex mixtures of unidentified byproducts (see Supporting Information (SI) for more details).

Scheme 1. Initial Attempts to Synthesize [¹¹C]SB-216763^a

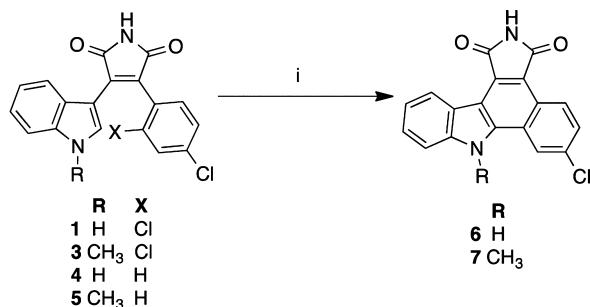


^aReagents and conditions: (i) 5 N KOH, EtOH, reflux, overnight (21%); (ii) [¹¹C]CH₃I, NaH, 45 °C, 4 min; (iii) LiHMDS, MeOH/DMF, 80 °C, 8 min.

We therefore abandoned this strategy and, in need of a new synthesis of [¹¹C]SB-216763, investigated the possibility of direct carbon-11 methylation of a maleimide precursor.

It has been reported that the lower pK_a of the maleimide NH (~ 10) when compared to that of the indole NH (~ 21) would favor ¹¹C-methylation at the maleimide if unprotected precursor **1** was radiolabeled directly.²⁷ However, given (1) that the indole NH is expected to be more nucleophilic than the maleimide NH because of the adjacent carbonyl groups on maleimide, (2) that treatment with methyl iodide is a classical strategy for methylating indoles,^{31,32} and (3) that a mixture of the two possible labeled products should be separable by semipreparative HPLC, we decided to test direct labeling of precursor **1** with [¹¹C]CH₃I (Scheme 1). Unfortunately, one radiolabeled product that did not correspond to [¹¹C]SB-216763 was obtained, as well as varying amounts of a nonradioactive side product. These labeling results were disappointing and somewhat perplexing. While the radioactive species was likely the labeled maleimide, the identity of the other nonradioactive side product was not clear. To understand the cause of these problems further, we exposed precursor **1** and unlabeled **3** to reaction conditions (NaH/[¹²C]CH₃I) on a larger scale so that stability of both the precursor and product to the reaction conditions, as well as the identity of any side products, could be examined using traditional analytical methods (NMR, mass spectrometry). To our surprise, in each case we obtained significant quantities of cyclized side products **6** or **7** (Scheme 2), which we expect arise from [3 + 3] sigmatropic shifts, followed by elimination of HCl to rearomatize.

To explore these pericyclic side reactions further, we performed an array of reactions subjecting both precursor **1** and reference standard **3** to a range of conditions (acid, base, heat) to understand the origins of the side products during labeling. Interestingly, cyclized products **6** and **7** were found in every case, albeit in varying amounts up to 32%. As a side note, we also subjected the monochloro unmethylated **4** and methylated **5** analogues to the matrix of conditions to identify whether the monochloro analogue of SB-216763 offered an alternate scaffold for radiolabeling that could circumvent this issue. Surprisingly, this did not improve the situation, and

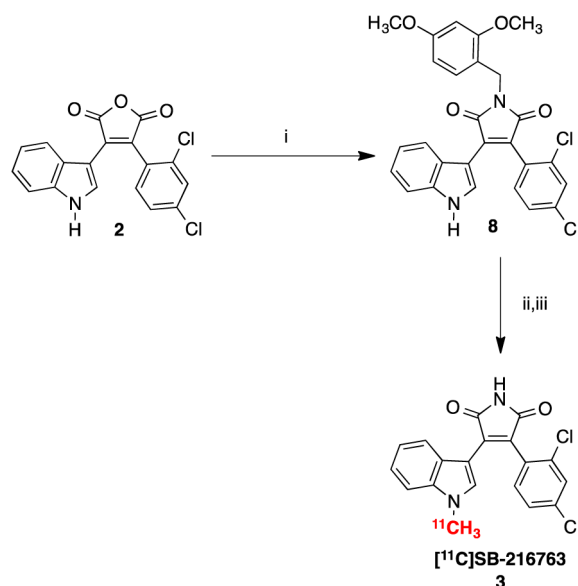
Scheme 2. [3 + 3] Sigmatropic Shifts^b

^bReagents and conditions: acid, base, or heat (see SI).

analogues 4 and 5 also underwent the [3 + 3] sigmatropic shift followed by elimination of H₂ to provide comparable yields of 6 and 7, respectively (see SI for full details of these experiments).

While pericyclic reactions of this kind are known in the literature for related compounds, they are normally mediated by light (in the presence of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DDQ, or iodine), or achieved using Pd-catalyzed Heck reactions.^{33–36} Such reactions being a complicating factor in this case were therefore surprising but nevertheless, it was clear that they were negatively impacting radiolabeling through consumption of precursor and/or base (6 was confirmed in the crude radiochemical reaction mixture (see SI), but we never observed formation of [¹¹C]7). We reasoned that inhibiting the [3 + 3] sigmatropic shift would improve ¹¹C-methylation. As the competing pericyclic side reaction is governed by strict orbital symmetry requirements, we postulated that it could be eliminated by disrupting the conformation of the molecule. Our strategy for this was to install a bulky protecting group on the maleimide nitrogen that could be easily removed under acidic conditions after radiolabeling. We initially considered installing a Boc group, but all of our attempts to Boc-protect 1 resulted in complex mixtures of products (cyclized products were again apparent in NMR spectra of crude reaction mixtures). We next considered using the bulky 2,4-dimethyl benzyl group as it can also be easily removed under acidic conditions, and the precursor (8) could be accessed from maleic anhydride 2, rather than maleimide 1. Thus, refluxing maleic anhydride 2 with 2,4-dimethoxy benzyl amine in acetic acid for 2 h yielded desmethyl precursor 8 in 79% yield, ready for radiolabeling (Scheme 3).

With the requisite precursor and reference standard in hand, we revisited the radiosynthesis of [¹¹C]SB-216763 (Scheme 3). [¹¹C]CO₂ (~3 Ci, 111 GBq) was produced in a cyclotron via the ¹⁴N(p,α)¹¹C nuclear reaction and delivered to the synthesis module where it was first reduced to [¹¹C]CH₄ by treating with hydrogen over a nickel catalyst at 350 °C. [¹¹C]CH₄ was then reacted with iodine at 750 °C to yield [¹¹C]CH₃I (~750 mCi, 27.8 GBq, ~26% yield from [¹¹C]CO₂). Gratifyingly, treatment of precursor 8 with NaH and subsequent methylation with [¹¹C]CH₃I provided protected [¹¹C]SB-216763. Subsequent deprotection proceeded smoothly upon treatment with 50% methanesulfonic acid solution in THF at 110 °C (Note: cleavage of the 2,4-dimethoxybenzyl group could not be accomplished using Ceric ammonium nitrate due to its poor solubility in THF). Purification of the crude reaction mixture by semipreparative HPLC yielded [¹¹C]SB-216763, which was reconstituted into ethanolic saline using an Oasis HLB sep-pak

Scheme 3. Radiosynthesis of [¹¹C]SB-216763 (3) from Precursor 8^c

^cReagents and conditions: (i) 2,4-dimethoxybenzyl amine, AcOH, 140 °C, 2 h (79%); (ii) [¹¹C]CH₃I, NaH, 45 °C, 4 min; (iii) 50% CH₃SO₃H in THF, 110 °C, 6 min (1% RCY).

cartridge to provide doses suitable for preclinical evaluation (radiochemical yields = 5–5.5 mCi, 185–200 MBq (1% noncorrected radiochemical yield (RCY) at end-of-synthesis (EOS) based upon [¹¹C]CH₃I), radiochemical purity = 97–100%, specific activity = 4–9 Ci/μmol, 150–350 GBq/μmol at EOS, *n* = 7).

Having successfully overcome our synthesis issues, we turned our attention to examining the imaging properties of [¹¹C]SB-216763 in rodents (Figure 2A) and nonhuman primates (Figure 2B) using preclinical PET imaging. Following imaging, regions-of-interest (ROIs) were drawn over brain regions on multiple planes of summed images. The volumetric ROIs were then applied to the full dynamic data set to generate time–radioactivity curves. Both rodent (Figure 2C) and nonhuman primate (Figure 2D) curves confirmed good initial brain uptake

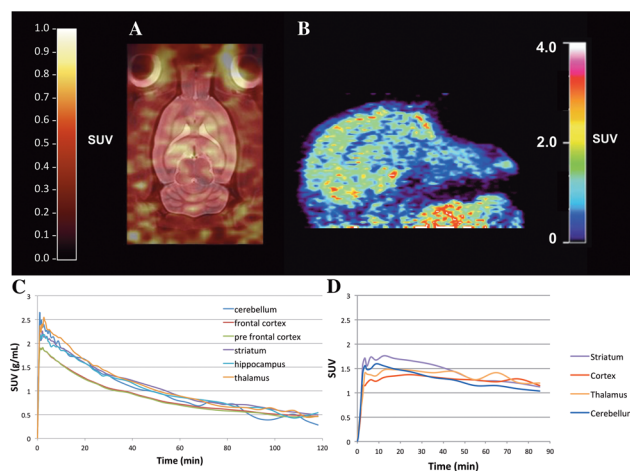


Figure 2. [¹¹C]SB-216763 data: (A) rodent SUV map (60–120 min p.i.); (B) summed primate PET image (0–90 min p.i.); (C) rodent time–radioactivity curves; (D) primate time–radioactivity curves.

(peak rodent standardized uptake value (SUV) = 2.5 @ 3 min postinjection (p.i.); peak primate SUV = 1.9 @ 5 min p.i. ($n = 2$)), followed by washout. Washout was faster in rodents than primates, and we attribute this to increased tissue volumes and lower blood flow in the primates. Brain uptake was fairly uniform throughout all brain regions in both species, which would be consistent with the ubiquitous distribution of GSK-3,³⁷ although the highest uptake was observed in thalamus, striatum, cortex, and cerebellum, areas of the mammalian brain known to be rich in GSK-3 β .³⁷

In summary, we have developed a novel one-pot two-step method for the radiosynthesis of [¹¹C]SB-216763 and demonstrated that it is the first radiotracer for GSK-3 able to cross the BBB and enter the CNS in both rodents and nonhuman primates. The arylindolemaleimide skeleton represents our lead scaffold for developing a radiotracer for quantification of GSK-3 *in vivo* using brain PET and ultimately translating it into clinical use.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental details and copies of NMR spectra and/or HPLC chromatograms for all compounds synthesized; HRMS for standards and precursors; procedures for radiochemical syntheses and quality control; protocols for animal PET imaging studies. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

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

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

BBB, blood–brain barrier; Boc, *tert*-butyloxycarbonyl; CNS, central nervous system; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; GSK-3, glycogen synthase kinase-3; HMDS, hexamethyldisilazane; HPLC, high-performance liquid chromatography; PET, positron emission tomography; p.i., post

injection; NMR, nuclear magnetic resonance; RCY, radiochemical yield; ROI, regions-of-interest; SUV, standardized uptake value; TLC, thin layer chromatography

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