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Synthesis and ex vivo evaluation of carbon-11 labelled *N*-(4-methoxybenzyl)-*N*′-(5-nitro-1,3-thiazol-2-yl)urea ([^{11}C]AR-A014418): A radiolabelled glycogen synthase kinase-3β specific inhibitor for PET studies

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Abstract—*N*-(4-Methoxybenzyl)-*N*′-(5-nitro-1,3-thiazol-2-yl)urea (AR-A014418), a highly selective inhibitor of glycogen synthase kinase-3β (GSK-3β), was radiolabelled with carbon-11 (half-life = 20.4 min) for cerebral positron emission tomography (PET) studies. Reaction of desmethyl AR-A014418 with [^{11}C]CH_3I produced [^{11}C]AR-A014418 in 17% decay-corrected radiochemical yield, based on [^{11}C]CO_2, with 3230 mCi/μmol specific activity after a 30 min synthesis time. The desmethyl precursor of AR-A014418 was synthesized in 23% yield by a novel one-pot reaction of 2-amino-5-nitrothiazole with in situ generated TMS-protected 4-hydroxybenzylisocyanate, following deprotection with acid. Ex vivo biodistribution studies were conducted after [^{11}C]AR-A014418 was administered via tail vein injection into Sprague–Dawley rats. Very low levels of radioactivity were found in all brain regions (0.08% injected dose/gram of tissue) at 5 and 30 min post-injection, uncorrected for vascular compartment. Considering the extremely poor brain penetration of [^{11}C]AR-A014418 this compound cannot be used to study GSK-3β in cerebral PET studies. Furthermore, the specific pharmacological mechanism(s) of antidepressant-like activity attributed to AR-A014418 should be investigated.

Glycogen synthase kinase 3β (GSK-3β) is a serine/threonine kinase with highest abundance in the brain[1] and is involved with signal transduction cascades of multiple cellular processes. GSK-3β facilitates a variety of complex functions including apoptotic mechanisms, neuronal plasticity, and gene expression.[2] Implications of dysregulated GSK-3β activity in certain psychiatric and neurodegenerative diseases have prompted investigations of GSK-3β inhibitors as therapeutics. Small molecule inhibitors of GSK-3β are currently under development for a broad range of illnesses including Alzheimer's disease, depression, diabetes, stroke, bipolar disorder and malignancy (for several recent reviews see Refs. 3–10).

One method for quantitation of enzyme levels in the living brain is by in vivo imaging with positron emission tomography (PET). Levels of GSK-3β levels could potentially be measured in the living animal and/or human brain by use of PET; however, there is no radiotracer available to achieve this as yet. It is the goal of this work to synthesize and evaluate the first radiopharmaceutical for imaging GSK-3β with PET. The highly selective inhibitor of GSK-3β, *N*-(4-methoxybenzyl)-*N*′-(5-nitro-1,3-thiazol-2-yl)urea (AR-A014418)[11] was chosen for radiolabelling with the positron emitting isotope carbon-11 (^{11}C; half-life = 20.4 min). This particular compound is purported to be a major advance in central nervous system (CNS) therapeutics and antidepressant development because previously developed inhibitors lacked selectivity for GSK-3β. Furthermore, a recent study has demonstrated antidepressant-like effects in rats exposed to a forced-swim test following administration of AR-A014418.[12]

The original synthetic route to [^{11}C]AR-A014418 involved the preparation of AR-A014418[11] and subsequent demethylation of AR-A014418 to serve as

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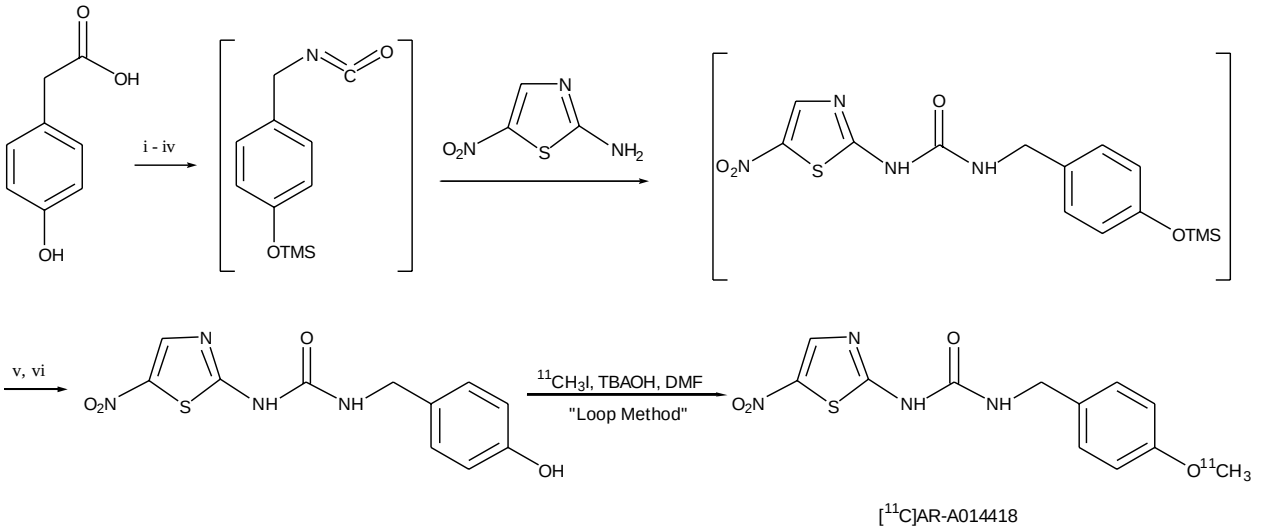
a precursor for radiolabelling with [^{11}C]iodomethane. While demethylations of anisoles to phenols are standard organic transformations,[13,14] cleavage of an aryl ether is difficult in the presence of a nitro-group.[15] In addition to containing a nitro-group, AR-A014418 presents a further complication as substituted 5-nitrothiazoles are highly susceptible to nucleophilic substitution.[16] A variety of demethylation conditions were attempted in the present work. Not surprisingly, all of them resulted in very low yields and/or halogenation at the thiazole moiety. For example, attempted demethylation of AR-A014418 using 5 M HBr in CH_3COOH[14] or 1 M BBr_3 in CH_2Cl_2[14] resulted in the formation of a demethylated and brominated derivative of AR-A014418 as the major product, likely *N*-(4-hydroxybenzyl)-*N*′-(5-bromo-1,3-thiazol-2-yl)urea, based on characterization by LC–MS.[17] Other attempts to cleave the ether group of AR-A014418 during this work included the use of $AlCl_3$/ethanethiol[14] and $AlCl_3$/pyridine/EtOAc[15] mixtures. While these procedures have been successful for cleavage of ethers in the presence of nitro-groups, reaction with AR-A014418 did not result in the desired demethylated product.

Alternatively, a novel one-pot synthesis of desmethyl AR-A014418 was successfully achieved by the reaction of in situ generated trimethylsiloxy-protected 4-hydroxybenzylisocyanate (prepared by modification of a previously described method[18]) with excess 2-amino-5-nitrothiazole, followed by acid deprotection (Scheme 1).[19] As shown in Scheme 1, reaction of 4-hydroxyphenylacetic acid with chlorotrimethylsilane generated the bis(silylated) hydroxyl acid, followed by conversion to 4-[(trimethylsilyl)oxy]-benzeneacetyl chloride with thionyl chloride. The acid chloride was then reacted with azidotrimethylsilane at room temperature to generate the intermediately formed acyl azide and was subsequently converted to the isocyanate upon heating the reaction mixture to reflux (Curtius rearrangement). Prior to refluxing the reaction mixture, excess 2-amino-5-nitrothiazole was added for reaction with the in situ generated isocyanate. Following deprotection with HCl and silica gel purification, desmethyl AR-A014418 was obtained in 23% overall yield.[19] This synthetic route is generally applicable for the syntheses of hydroxybenzyl-ureas and offers an alternative to problematic dealkylation reactions.

For the radiosynthesis of [^{11}C]AR-A014418, desmethyl AR-A014418 was dissolved in DMF and deprotonated with tetrabutylammonium hydroxide (TBAOH) immediately prior to reaction with [^{11}C]CH_3I, using the 'Loop' method[20] (Scheme 1). Following methylation, [^{11}C]AR-A014418 was purified by preparative HPLC and formulated in saline.[21] Full-scale production of [^{11}C]CO_2 resulted in 83 mCi [^{11}C]AR-A014418, ready for injection, corresponding to 17% decay-corrected radiochemical yield, based on [^{11}C]CO_2, with a specific activity of 3230 mCi/μmol at the end of synthesis.[21]

Ex vivo biodistribution studies following administration of high specific activity [^{11}C]AR-A014418 in male Sprague–Dawley rats (250 ± 8 g) were conducted as previously described by our group.[22,23] Rats were injected in the tail vein with a saline solution of [^{11}C]AR-A014418 (0.7 μg/kg) and sacrificed at 5 and 30 min post-injection ($n = 6$ per time point) and brain regions were excised and measured for radioactivity. Blood (trunk) was also collected for radioactivity measurement. Figure 1 shows the distribution of activity for [^{11}C]AR-A014418 in eight regions of the rat brain known to contain glycogen synthase kinase-3β,[24] the remainder of the brain, and whole blood. The average percent injected dose per gram (% ID/g) of wet tissue in the brain was 0.08% ID/g for all regions at time points of 5 and 30 min. Whole blood levels of [^{11}C]AR-A014418 were 0.84% and 0.46% ID/g at 5 and 30 min, respectively. When corrected for radioactivity in the vascular compartment,[25] uptake values of radioactivity are further reduced from those shown in



Scheme 1. Synthesis of desmethyl AR-A014418 and [^{11}C]AR-A014418. Reagents and conditions: (i) TMS-Cl, Et_3N, toluene, reflux; (ii) $SOCl_2$, CH_2Cl_2, reflux; (iii) TMS-N_3, dioxane, rt; (iv) reflux (Curtius rearrangement); (v) 2 N HCl; (vi) silica gel purification.

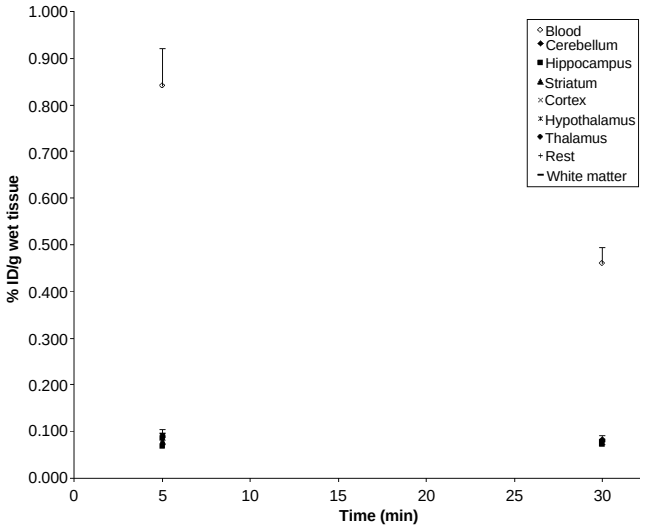


Figure 1. Distribution of radioactivity in rat brain regions and whole blood at 5 and 30 min ($n = 6$ per time point; means ± SD shown) following tail vein injection of [^{11}C]AR-A014418 is shown. Homogeneous distribution of low levels of radioactivity was seen in all brain regions.

Figure 1 to 0.04% and 0.06% ID/g of wet tissue at 5 and 30 min, respectively. Thus, the brain is almost totally impermeable to this compound (successful radiotracers for imaging the central nervous system typically express a % ID/g of tissue $\geqslant$0.5% in rodent brain[26]).

Ex vivo measurement of radioactivity in tissue after injection of [^{11}C]AR-A014418 in rats clearly indicates that this compound cannot be used to study GSK-3β in the CNS with PET. The lack of brain penetration of [^{11}C]AR-A014418 is surprising considering that its log*P* value between 1-octanol and 0.02 M phosphate buffer at pH 7.4 was found to be 2.44, using a previously described procedure,[27] a value which is typical of many brain penetrating compounds.[27,28] The lack of brain penetration is also surprising in light of reported CNS effects following peripheral administration of AR-A014418 into rats.[12] Studies to investigate peripheral effects, metabolism and brain barrier transport of AR-A014418 are presently underway in our laboratory and hope to gain further insight into the pharmacological mechanism(s) by which this compound could exert its reported antidepressant-like effects.[12]

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17. LC–MS was conducted by Electrospray Ionization using a C-18 Zorbax column (50 mm × 2.1 mm, 3.5 μm) eluted with CH_3CN/H_2O (40:60 v/v) + 0.5% formic acid using a flow rate of 250 μL min^{-1} in conjunction with a MDS Sciex QStar mass spectrometer. A desmethyl AR-A014418 derivative with substitution at the thiazole moiety is predicted based on the parent molecular ion for this compound which showed a characteristic isotopic pattern for monobromination, [M+H]: *m*/*z* = 328 and 330 (1:1). The hydroxybenzyl moiety was seen at *m*/*z* = 107 in addition to pairs of fragments corresponding to brominated thiazolamine, [M+H]: *m*/*z* = 179 and 181 (1:1). A trace of dibrominated product was also seen, [M+H]: *m*/*z* = 406, 408, and 410 (1:2:1), respectively, with both the thiazole and benzyl fragments containing a bromine. Time-course of this reaction revealed that bromination at the thiazole occurs (<15 min) prior to demethylation (ca. 1 h) therefore, this synthetic route was no longer pursued.
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AR-A014418 was obtained as a yellow solid in 23% overall yield. ^{1}H NMR (Me_2SO-d_6, 499.8 MHz) δ (ppm, relative to TMS): 11.9 (br s, $\Delta\nu_{1/2}$ = 119 Hz, 1H,), 9.34 (br s, $\Delta\nu_{1/2}$ = 2.5 Hz, 1H), 8.50 (s, 1H), 7.19 (br t, $\Delta\nu_{1/2}$ = 13.9 Hz, J = ca. 5.5 Hz, 1H; confirmed to be N*H*–CH_2Ph by COSY), 7.11 (AA′ XX′, J_A = 3.5 Hz, J_X = 1.4 Hz, J = 8.3 Hz, J' = −0.3 Hz, 2H), 6.72 (AA′ XX′, J_A = 3.5 Hz, J_X = 1.4 Hz, J = 8.3 Hz, J' = −0.3 Hz, 2H), 4.24 (d, J = 5.8 Hz, 2H). ^{13}C NMR (Me_2SO-*d*6, 125.7 MHz) δ (ppm, relative to TMS): 64.3, 156.5, 153.3, 143.3,140.8, 128.9, 128.7, 115.1, 42.7. HRMS (ESI) calcd for $C_{11}H_{11}N_4O_4S$, 295.0495; found: 295.0471 (M^++H).

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21. Radiosynthesis was shown to be reliable and efficient (30 min) for the production of mCi quantities of chemically and radiochemically pure [^{11}C]AR-A014418 (>98%; n = 5). In a full-scale production of [^{11}C]CO_2, 1% O_2 in N_2 was bombarded in a 17 MeV cyclotron with 45 μA proton beam current for 20 min. Desmethyl AR-A014418 (1.0 mg, 3.4 μmol) was dissolved in 75 μL DMF and 6.5 μL of a 1.0 M solution of tetrabutylammonium hydroxide (TBAOH) in methanol was added prior to a 5 min at room temperature reaction with [^{11}C]iodomethane using the 'Loop' method.[20] Reverse-phase semi-preparative HPLC (Phenomenex Prodigy ODS-Prep, 250× 10 mm, 10 μm) was performed using a mobile phase consisting of CH_3CN/H_2O (50:50 v/v) + 0.1 N ammonium formate (AF) at a flow rate of 7 mL min^{-1} to separate [^{11}C]AR-A014418 (t_R = 5.9 min) from unreacted desmethyl AR-A014418 (t_R = 3.1 min) and undesired byproducts. The reaction mixture was monitored during purification and analysis using an in-line UV (λ = 254 nm) detector in series with a NaI crystal radioactivity detector. The product eluting at 5.9 min was led into a rotary evaporation flask containing 250 μL ascorbic acid solution (40 mg mL^{-1} in H_2O) and heated to dryness at 70 °C, under vacuum. The product was dissolved in 10 mL saline and transferred into a vial containing 1 mL of 8.4% sodium bicarbonate. The pH of the final solution was between 7 and 8. Analytical HPLC was performed using a C-18 column (Phenomenex Prodigy ODS-Prep, 250 mm × 4.6 mm, 10 μm) eluted with CH_3CN/H_2O (40:60 v/v) + 0.1 N AF using a flow rate of 4 mL min^{-1}. Authentic AR-A014418 (Calbiochem) co-eluted with the ^{11}C-labelled product under these conditions (t_R = 5.2 min). In addition, three other HPLC conditions were used to confirm the co-elution of authentic AR-A014418 and [^{11}C]AR-A014418: (1) analytical C-18 Prodigy column eluted with THF/H_2O (50:50 v/v) + 0.1 N AF using a flow rate of 3.5 mL min^{-1}; t_R = 2.1 min; (2) analytical C-18 Prodigy column eluted with CH_3CN/H_2O (40:60 v/v) + 0.1 N AF + 0.5% formic acid using a flow rate of 4 mL min^{-1}, t_R = 4.7 min; (3) Econosil C-8 column (Alltech; 250 mm × 4.6 mm,10 μm) eluted with CH_3CN/H_2O (40:60 v/v) + 0.1 N AF using a flow rate of 4 mL min^{-1}, t_R = 3.3 min.
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