Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2101–2104

Synthesis, in vitro and in vivo evaluation of [*O*-methyl-^{11}C] 2-{4-[4-(3-methoxyphenyl)piperazin-1-yl]-butyl}-4-methyl-2*H*-[1,2,4]-triazine-3,5-dione: A novel agonist 5-HT$_{1A}$ receptor PET ligand

Jaya Prabhakaran,[a] Ramin V. Parsey,[a,c] Vattoly J. Majo,[a] Shu-Chi Hsiung,[c] Matthew S. Milak,[a] Hadassah Tamir,[a,c] Norman R. Simpson,[b] Ronald L. Van Heertum,[b,c] J. John Mann[a,b,c] and J. S. Dileep Kumar[a,c,*]

[a]*Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York State Psychiatric Institute, New York, NY 10032, USA*
[b]*Department of Radiology, Columbia University College of Physicians and Surgeons, New York State Psychiatric Institute, New York, NY 10032, USA*
[c]*Department of Neuroscience, New York State Psychiatric Institute, New York, NY 10032, USA*

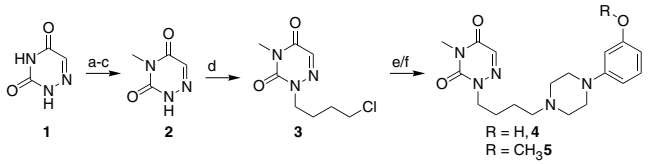
Received 6 December 2005; revised 17 January 2006; accepted 17 January 2006
Available online 3 February 2006

Abstract—Synthesis and in vivo evaluation of 2-{4-[4-(3-methoxyphenyl)piperazin-1-yl]-butyl}-4-methyl-2*H*-[1,2,4]triazine-3,5-dione (**5** or MMT), a high affinity and selective serotonin 5-HT$_{1A}$R agonist PET tracer, are described. GTPγS assay shows that MMT is an agonist with an EC$_{50}$ comparable to 5-HT. Radiolabeling of **5** was achieved in 30% yield (EOS) from desmethyl-MMT (**4**) with >99% chemical and radiochemical purities and a specific activity >1000 Ci/mmol. PET studies in baboon show that [^{11}C]**5** penetrates the blood–brain barrier but, because of low specific binding and fast clearance of radioactivity it is not a suitable PET tracer for the in vivo quantification of 5-HT$_{1A}$R.

The serotonin$_{1A}$ receptor (5-HT$_{1A}$R) has been implicated in the pathophysiology of major psychiatric and neurological disorders and the action of psychotropic medications such as antidepressants.[1–6] Successful radioligands studied to date for 5-HT$_{1A}$R are antagonists ligands such as [carbonyl-^{11}C]WAY100635 (WAY) [carbonyl-^{11}C]desmethyl-WAY100635 (DWAY) or *p*-[^{18}F]MPPF.[7–9] The major limitation of using antagonist tracers in imaging the 5-HT$_{1A}$R is that these have comparable affinity to both the G-protein-coupled high affinity state and uncoupled low affinity state of 5-HT$_{1A}$R. In contrast, agonist PET radiotracers bind preferentially to the HA state of the receptor, thereby providing a more meaningful functional measure of 5-HT$_{1A}$R.[10–12] An agonist tracer may also be more sensitive to changes in endogenous serotonin concentrations and allow measurement of 5-HT$_{1A}$R occupancy by agonist drugs. We have recently reported [^{11}C]MPT as a 5-HT$_{1A}$R agonist PET ligand and this has been evaluated in baboons.[13] Parallel to these studies, structural variants of MPT were examined in order to find an agonist radioligand possessing more favorable kinetics to quantify 5-HT$_{1A}$R in vivo. Here, we report the synthesis and evaluation of 2-{4-[4-(3-methoxyphenyl)piperazin-1-yl]butyl}-4-methyl-2*H*-[1,2,4]triazine-3,5-dione (**5** or MMT), a phenyl analogue of MPT as an agonist PET ligand for 5-HT$_{1A}$R. We have synthesized **5** from 6-azaurazil in 5 steps (Scheme 1). Synthesis of the intermediate 4-methyl-2*H*-[1,2,4]triazine-3,5-dione (**2**) was achieved in 76% yield by treating acetylated 6-azaurazil with methyl iodide in the presence of sodium hydride followed by deprotection with *p*-TsOH. Substitution of 4-chlorobutyl group in compound **2** was achieved in 70% yield by the addition of 1-bromo-4-chlorobutane in the presence of sodium hydride. The synthesis of MMT (**5**) has been accomplished by the condensation of 2-(4-chlorobutyl)-4-methyl-2*H*-[1,2,4]-triazine-3,5-dione (**3**) with 1-(3-methoxyphenyl)piperazine in 86%

Keywords: [^{11}C]MMT; Positron emission tomography; Agonism; Radiotracer.
* Corresponding author. Fax: +1 212 543 1054; e-mail: dk2038@columbia.edu

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.01.052



Scheme 1. Synthesis of **5** and desmethyl-MMT. Reagents and conditions: (a) $(CH_3CO)_2O$ (6 equiv), 130 °C, 1 h; (b) NaH, DMF, CH_3I, rt, 12 h; (c) *p*-TsOH, EtOH, 70 °C (3 steps, 85%); (d) NaH, DMF, $Br(CH_2)_4Cl$, 12 h, 75%; (e) 3-piperazin-1-ylphenol, TEA, BuOH, 70 °C, 75%; (f) 1-(3-methoxyphenyl)piperazine, TEA, BuOH, 70 °C, 86%.

yield. Under identical conditions, synthesis of desmethyl-MMT (**4**) was achieved by reacting **3** with 3-piperazin-1-yl-phenol in 75% yield.[14]

The affinity (K_i) and selectivity of **5** has been determined by radioligand binding assays through NIMH-psychoactive Drug Screening Program (PDSP). Results show that **5** has a K_i value of 1.1 nM for the 5-HT_{1A}R and has no appreciable affinity for a variety of biogenic amines, receptors, and transporters (Table 1).

We examined the agonist properties of **5** on 5-HT_{1A}R using [^{35}S]GTPγS binding in membranes of Chinese hamster ovary cells stably expressing the human 5-HT_{1A}R (CHO-h5-HT_{1A} cells).[15] Figure 1 shows the dose–response curves for 5-HT_{1A}R stimulated

Table 1. In vitro binding data of **5**

Target	K_i (nM)	Target	K_i (nM)
5-HT_{1A}	1.1 ± 0.09	5-HT_6	>10,000
5-HT_{2B}	33.7 ± 12.8	5-HT_{5a}	>10,000
Alpha $_{2C}$	109 ± 19	DOR	>10,000
Sigma1	180 ± 44	NET	>10,000
5-HT_{1B}	228 ± 77	AMPA	>10,000
Alpha $_{2A}$	250 ± 75	VMAT	>10,000
5-HT_7	328 ± 45	GABA	>10,000
Alpha$_{1B}$	378 ± 15	BZP	>10,000
Alpha $_{1A}$	413 ± 25	KO-R	>10,000
H_1	839 ± 90	MDR_1	>10,000
5-HT_{2A}	2100	EP	>10,000
H_2	2126	5-HT_{1E}	>10,000
D_2	2575	D_3	>10,000
D_5	>10,000	D_4	>10,000
A_1,A_2,A_3, A_4	>10,000	H_3, H_4	>10,000
HERG	>10,000	KA-R	>10,000
5-HT_3	>10,000	M	>10,000
mGluR	>10,000	NMDA	>10,000
NK	>10,000	NT	>10,000
Ca^+, Na^+ channels	>10,000	V	>10,000
DAT	>10,000	SERT	>10,000

A, adenosine; Alpha Beta; BZP, benzodiazepine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; V, vasopressin; CB, cannabinoid; D, dopamine; DAT, dopamine transporters; DOR: delta opioid receptors; EP, prostanoid receptors: GABA, Gamma-amino butyric acid; H, histamine; hERG, *Human Ether-a-go-go*; KOR, kappa opioid receptors; M, muscarinic; MDR, multidrug resist; MOR, mu opioid receptor; mGluR, metabotropic glutamate receptors; NMDA, *N*-methyl-D-aspartic acid; NK, neurokinin; SERT, serotonin transporter; VMAT, vesicular monoamine transporter; NET, norepinephrine transporter; NT, neurotrophin.

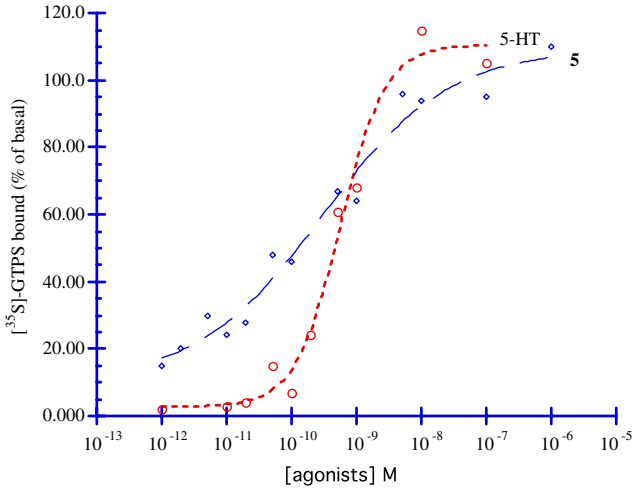
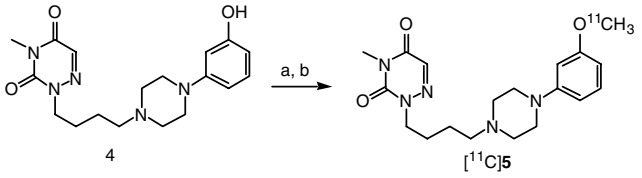


Figure 1. Effect of 5-HT_{1A}R agonist concentration on the stimulation of [^{35}S]GTPγS binding in CHO cells. Values are shown as expressed as a percentage above basal which is the binding of [^{35}S]GTPγS in the absence of agonists. Data points are means of duplicate determinations from representative experiments repeated on at least three independent occasions with similar results.

[^{35}S]GTPγS binding assay for **5** and 5-HT. The dose–response curves show an increased binding of [^{35}S]GTPγS with respect to the basal level. The maximum level of agonist stimulated binding of [^{35}S]GTPγS is comparable for **5** and 5-HT with an E_{max} 95% for **5** relative to 5-HT. The dose–response curve of MMT is also comparable to that of 5-HT with an EC_{50} of 0.3 and 0.7 nM, respectively. These results show that **5** is a 5-HT_{1A}R agonist with intrinsic activity similar to that of 5-HT. 5-HT, unlike **5**, showed a steeper slope at a lower concentration of the ligand.

Compound **5** was obtained by methylation of **4** using [^{11}C]MeOTf using standard radiolabeling procedures for phenols, that we previously optimized.[16] Optimum yields were obtained by treating **4** with [^{11}C]MeOTf in the presence of NaOH (Scheme 2).[17] The crude product was purified by reverse-phase HPLC followed by C-18 Sep-Pak® purification to obtain [^{11}C]**5** in 30% yield at the end of synthesis (EOS) (n = 4, SD = ±5). The formation of [^{11}C]**5** was confirmed by co-injecting the [^{11}C]-product with non-radioactive compound and comparing the HPLC retention times of the two compounds. Multiple mobile phases were used for determining the purities of the radioligand.[17] Specific activity obtained for [^{11}C]**5** was 1400 Ci/mmol (n = 3, SD = ±300) based on a standard mass curve.



Scheme 2. Radiosynthesis of [^{11}C]**5**. Reagents and conditions: (a) acetone, NaOH, [^{11}C]MeOTf, rt, 5′, heat 70 °C, 2′; (b) semi-PREP HPLC.

PET studies were conducted in adult baboons using [^{11}C]**5**.[18] The reconstructed brain image shows that [^{11}C]**5** enters brain (Figure 2) however time–activity curves (TACs) reveal very little retention of radioactivity in 5-HT$_{1A}$R specific regions (Figure 3). The metabolite analyses show that [^{11}C]**5** undergoes fast metabolism and polar metabolites were found in baboon plasma and the percentage of unmetabolized fractions were 92%, 87%, 52%, 32%, 22%, and 10% at 2, 4, 12, 30, 60, and 90 min, respectively.[19] The lack of specificity of [^{11}C]**5** in baboon brain is possibly due to its metabolism in brain.

In summary, we synthesized **5**, an agonist ligand with a K_i value of 1.1 nM and intrinsic agonist activity comparable to those of 5-HT. The radiosynthesis of [^{11}C]**5** has been achieved with 30% yield (EOS), high purity, and specific activity. PET studies in anesthetized baboon show that [^{11}C]**5** penetrates the blood–brain barrier. Kinetic studies of the radiotracer in baboon demonstrate a fast washout as well as no localized distribution of ligand binding, indicating minimal specific binding. The lack of specific binding is unexpected given the comparable K_i values of [^{11}C]**5** for 5-HT$_{1A}$R (1.1 nM) and the structurally identical [^{11}C]MPT (K_i = 1.36 nM), which specifically binds to 5-HT$_{1A}$R regions.[13] However, a structure–activity relationship study of **5** may provide a better PET ligand with in vivo kinetics that permit valid and reliable measurement of 5-HT$_{1A}$R binding.

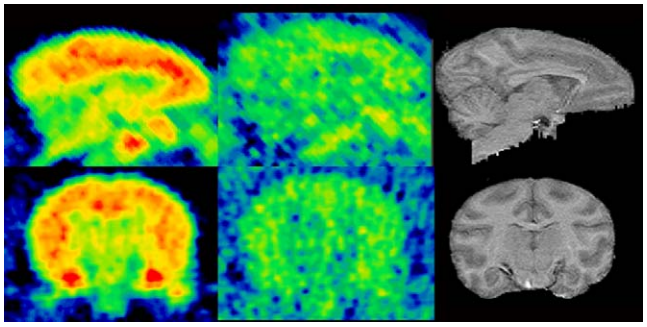


Figure 2. PET and MRI images of [^{11}C]**5** in baboon brain (PET images are normalized to the injected dose). (First column) Sum of 14–40 min PET images; (Second column) sum of last 60 min PET images; (Last column) MRI; (Top row) sagittal; (Bottom row) coronal views.

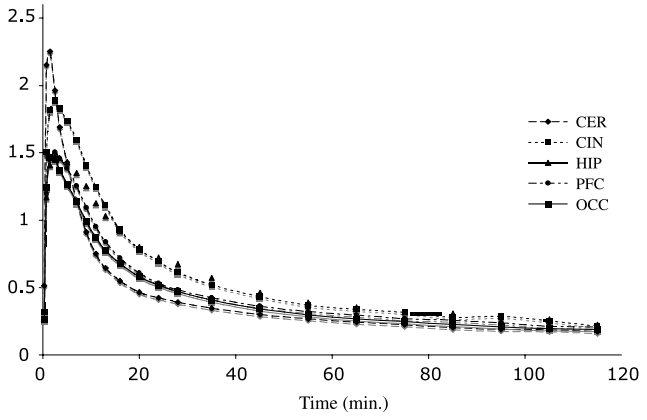


Figure 3. Time activity curves of the radiotracer in baboon after the injection of [^{11}C]**5**. CER, cerebellum; CIN, cingulate; HIP, hippocampus; PFC, prefrontal cortex; OCC, occipital cortex.

Acknowledgments

The authors thank Dr. Bryan Roth and the NIMH-PDSP program for the competitive receptor-transporter binding assays. The authors also thank Ms. Monica Chung for her assistance in the radiolabeling studies.

References and notes

1. Arango, V.; Underwood, M. D.; Boldrini, M.; Tamir, H.; Kassir, S. A.; Hsiung, S.-C.; Chen, J. J. X.; Mann, J. J. *Neuropsychopharmacology* **2001**, *25*, 892.
2. Arango, V.; Huang, Y. Y.; Underwood, M. D.; Mann, J. J. *J. Psychiatr. Res.* **2003**, *37*, 375.
3. Sullivan, G. M.; Oquendo, M. A.; Simpson, N.; Van Heertum, R. L.; Mann, J. J.; Parsey, R. V. *Biol. Psychiatry* **2005**, *58*, 947.
4. Frazer, A.; Maayani, S.; Wolfe, B. B. *Annu. Rev. Pharmacol. Toxicol.* **1990**, *30*, 307.
5. Lai, M. K. P.; Tsang, S. W. Y.; Francis, P. T.; Esiri, M. M.; Keene, J.; Hope, T.; Chen, C. P. L. H. *Brain Res.* **2003**, *974*, 82.
6. Meltzer, H. Y.; Li, Z.; Kaneda, Y.; Ichikawa, J. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2003**, *27*, 1159.
7. Tauscher, J.; Verhoeff, N. P. L. G.; Christensen, B. K.; Hussey, D.; Meyer, J. H.; Kecojevic, A.; Javanmard, M.; Kasper, S.; Kapur, S. *Neuropsychopharmacology* **2001**, *24*, 522.
8. Pike, V. W.; Halldin, C.; McCarron, J. A.; Lundkvist, C.; Hirani, E.; Olsson, H.; Hume, S. P.; Karlsson, P.; Osman, S.; Swahn, C.-G.; Hall, H.; Wikstrom, H.; Mensonidas, M.; Poole, K. G.; Farde, L. *Eur. J. Nucl. Med.* **1998**, *25*, 338.
9. Zimmer, L.; Rbah, L.; Giacomelli, F.; Le Bars, D.; Renaud, B. *J. Nucl. Med.* **2003**, *44*, 1495.
10. Clawges, H. M.; Depree, K. M.; Parker, E. M.; Graber, S. G. *Biochemistry* **1997**, *36*, 12930.
11. Watson, J.; Collin, L.; Ho, M.; Riley, G.; Scott, C.; Selkirk, J. V.; Price, G. W. *Br. J. Pharmacol.* **2000**, *130*, 1108.
12. Gozlan, H.; Ponchant, M.; Daval, G.; Verge, D.; Menard, F.; Vanhove, A.; Beaucourt, J. P.; Hamon, M. *J. Pharmacol. Exp. Ther.* **1988**, *244*, 751.
13. Kumar, J. S. D.; Majo, V. J.; Hsuing, S.-C.; Milak, M. S.; Liu, P.; Tamir, H.; Prabhakaran, J.; Simpson, N. R.; Van Heertum, R. L.; Mann, J. J.; Parsey, R. V. *J. Med. Chem.* **2006**, *49*, 125.
14. A solution of 1-(3-methoxyphenyl)piperazine (150 mg, 0.8 mmol) and azaurazil derivative **3** (183 mg, 0.8 mmol) was dissolved in 1-butanol (5 mL) and treated dropwise with Et_3N (0.5 mL). The reaction mixture was refluxed for 12 h and then concentrated under vacuum. The residue was column chromatographed over silica gel (4% MeOH in $CHCl_3$) to give **5** (250 mg, 86%) as a colorless solid. **5**: ^{1}H NMR ($CDCl_3$, 400 MHz): 7.30 (s, 1H), 7.14 (t, 1H), 6.44 (m, 3H), 4.0 (t, 2H), 3.77 (s, 3H), 3.33 (m, 3H), 3.18 (m, 4H), 2.58 (m, 4H), 2.42 (m, 2H), 1.80–1.57 (m, 4H): HRMS (EI^+) calcd for $C_{19}H_{28}O_3N_5$ 374.2192; Found: 374.2181 Under identical conditions coupling of **3** with 3-piperazin-1-ylphenol provided desmethyl-MMT (**4**) in

75% yield. **4**: ^{1}HNMR ($CDCl_3$, 400 MHz): 7.36 (s, 1H), 7.05 (t, 1H), 6.32 (m, 3H), 3.99 (t, 2H), 3.33 (s, 3H), 3.15 (m, 4H), 2.59 (m, 4H), 2.43 (m, 2H), 1.78 (m, 2H), 1.57 (m, 2H): HRMS (EI^+) calcd for $C_{18}H_{26}O_3N_5$ 360.2036; Found: 360.2033.

15. *Agonist stimulated [^{35}S]GTPγS binding assay*. These experiments were carried out as described previously with some modification by Newman-Tancredi, A. et al. *Mol. Pharmacol.* **2002**, *62*, 590–601. CHO-h5-HT_{1A} membranes (30 μg) were preincubated with agonists for 5 min at room temperature with indicated concentrations in a buffer containing 20 mM HEPES, pH 7.4, 3 mM $MgCl_2$, 100 mM NaCl, and 3 μM GDP in a final volume of 0.5 mL. [^{35}S]GTPS (0.1 nM; 1250 Ci/mmol Perkin Elmer Life Science, Boston, MA) was added and the incubation was continued for 60 min at rt. Experiments were terminated by rapid filtration through Whatman GF/B filters followed by three washes with ice-cold 20 mM Hepes buffer, pH 7.4, using a cell harvester. Bound radioactivity was determined by liquid scintillation spectrometry (Beckman).
16. Kumar, J. S. D.; Prabhakaran, J.; Arango, V.; Parsey, R. V.; Underwood, M. D.; Simpson, N. R.; Kassir, S. A.; Majo, V. J.; Van Heertum, R. L.; Mann, J. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2393.
17. Radiosynthesis of [^{11}C]**5**: The precursor desmethyl-MMT **4** (1.0 mg) was dissolved in 500 μL acetone in a capped 5 mL V-vial. Sodium hydroxide (10 μL, 5 M) was added and the resultant solution allowed to stand for 2 min. High specific active [^{11}C]CO_2 produced from RDS112 Cyclotron (~37 GBq) was subsequently converted into [^{11}C]CH_3OTf and transported by a stream of argon (20–30 mL/min) into the vial over approximately 5 min at room temperature. At the end of the trapping, the product mixture was diluted with 0.5 ml acetonitrile and was directly injected into a semipreparative RP-HPLC (Phenomenex C18, 10 × 250 mm, 10 μ) and eluted with acetonitrile/ 50 mM sodium phosphate solution (45:55) at a flow rate of 10 mL/min. The precursor appeared after 4–5 min retention time during the HPLC analysis. The product fraction with a retention time of 8–9 min based on a γ-detector was collected, diluted with 100 mL of deionized water, and passed through a classic C-18 Sep-Pak® cartridge. Reconstitution of the product in 1 mL of absolute ethanol afforded [^{11}C]**5** (30% yield, based on $^{11}CH_3I$ at EOS). A portion of the ethanol solution was analyzed by analytical RP-HPLC (Phenomenex, Prodigy ODS(3) 4.6 × 250 mm, 5 μ; mobile phase: acetonitrile/ 50 mM sodium phosphate solution (50:50), flow rate: 2 mL/min, retention time: 8 min, wavelength: 240 nm) to determine the specific activity and radiochemical purity. The radiochemical purity was further confirmed by RP-HPLC (Phenomenex, Prodigy ODS(3) 4.6 × 250 mm, 5 μ; mobile phase: acetonitrile/0.1 M ammonium formate/triethylamine (35:64.5:0.5), flow rate: 2 mL/min, retention time: 6.4 min, wavelength: 256 nm). Then the final solution of the [^{11}C]**5** in 10% ethanol-saline was analyzed to confirm the chemical and radiochemical purities.
18. Baboon PET scanning with [^{11}C]**5**: fasted animals were immobilized with ketamine (10 mg/kg, im) and anesthetized with 1.5–2.0% isoflurane via an endotracheal tube. Core temperature was kept constant at 37 °C with a heated water blanket. An iv infusion line with 0.9% NaCl was maintained during the experiment and used for hydration and radiotracer injection. An arterial line was placed for obtaining arterial samples for the input function. After a 10 min transmission scan, 5 ± 0.5 mCi of [^{11}C]**5** (S.A. of 1400 ± 300 Ci/mmol) was injected as an iv bolus and emission data were collected for 120 min in 3-D mode in a SiemensECAT EXACT HR+ (CPS/Knoxville, TN). The head was positioned at the center of the field of view as defined by imbedded laser lines. Regions of interest drawn on the animal's MRI scan were transferred to co-registered (AIR) frames of PET data.
19. The percentage of radioactivity in plasma as unchanged [^{11}C]**5** was determined by the HPLC method. Blood samples were taken at 2, 4, 12, 30, 60, and 90 min after radiotracer injection for metabolite analyses. The supernatant liquid (0.5 ml) obtained after centrifugation of the blood sample at 2000 rpm for 1 min was transferred (0.5 mL) into a tube and mixed with acetonitrile (0.7 mL). The resulting mixture was vortexed for 10 s and centrifuged at 14,000 rpm for 4 min. The supernatant liquid (~1 mL) was removed, the radioactivity measured in a well counter and the majority (0.8 mL) was subsequently injected onto the HPLC column and eluted with a mobile phase; acetonitrile/50 mM sodium phosphate solution (45:55) at 2 ml/min (Phenomenex, Prodigy ODS(3), C18, 4.6 × 250 mm, 5 μ). The metabolite and free fractions (6.3 min) were collected using a Bioscan gamma detector and all the acquired data were then subjected to correction for background radioactivity and physical decay to calculate the percentage of [^{11}C]**5** in the plasma at different time points.