Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 24 February 2014, Revised 9 April 2014, Accepted 11 April 2014 Published online 3 July 2014 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3206

Synthesis and initial preclinical evaluation of the $P2X_7$ receptor antagonist [^{11}C]A-740003 as a novel tracer of neuroinflammation

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Neuroinflammation, in particular activation of microglia, is thought to play an important role in the progression of neurodegenerative diseases. In activated microglia, the purinergic $P2X_7$ receptor is upregulated. A-740003, a highly affine and selective $P2X_7$ receptor antagonist, is a promising candidate for the development of a radiotracer for imaging of neuroinflammation by positron emission tomography. For this purpose, [^{11}C]A-740003 was synthesised and evaluated *in vivo* with respect to both tracer metabolism and biodistribution. In plasma, a moderate metabolic rate was seen. In healthy rat brain, only marginal uptake of [^{11}C]A-740003 was observed and, therefore, metabolites in brain could not be determined. Whether the minimal brain uptake is due to the low expression levels of the $P2X_7$ receptor in healthy brain or to limited transport across the blood–brain barrier has yet to be elucidated.

Keywords: radiolabelling; carbon-11; A-740003; $P2X_7$ receptor; neuroinflammation; positron emission tomography

Introduction

Neuroinflammation is thought to play a key role in the progression of several pathologies of the central nervous system (CNS), including Alzheimer's disease, Parkinson's disease, multiple sclerosis and amyotrophic lateral sclerosis.[1–3] Microglia, as resident macrophages of the CNS, are involved in the neuroinflammatory response.[2,4] Microglia switch from a resting to an activated state in response to, for example, tissue damage, pathogen invasion and protein aggregates. Next to their involvement in the immune response to clear pathogens and promote tissue repair, excessive activity of microglia may also contribute to the progression of CNS pathologies.[2,5] Activated microglia have been found in affected brain areas of various pathologies and, therefore, they may be a viable diagnostic marker for disease onset or progression.[5,6] Following their activation, microglia alter the expression of, amongst others, cell surface receptors and intracellular signalling molecules. One of the cell surface receptors with upregulated expression in the activated state of microglia is the purinergic $P2X_7$ receptor.[2,6] This receptor is thought to play a minimal role in normal physiology but has been shown to induce the release of inflammatory and bioactive substances, which denote microglial activation.[2,7]

The $P2X_7$ receptor comprises two transmembrane domains, an extracellular loop (with the adenosine triphosphate (ATP) binding site) and *N*-terminal and *C*-terminal domains. Short stimulation by binding of agonists (e.g. ATP) leads to formation of a channel permeable to small cations, whereas prolonged agonistic stimulation leads to a pore state that permits passage of molecules up to 900 Da. Monif et al.[2] proposed a model for the involvement of $P2X_7$ receptors in the neuroinflammatory response. Upon binding of ATP to the receptor, microglia are activated and proliferation of microglia is stimulated. Through the pore that is then formed, chemokines are released to recruit other microglia. Release of proinflammatory cytokines (e.g. interleukin-1β (IL-1β) and tumour necrosis factor α (TNFα)) through the pore leads to further microglial activation. Furthermore, IL-1β and TNFα are shown to promote neuronal damage, and subsequent apoptosis leads to increased ATP release. Continued presence of ATP and an increasing level of proinflammatory cytokines maintain the cycle of degeneration and inflammation.[2] According to this model, a $P2X_7$ receptor antagonist might halt the inflammatory cascade and thus further progression of neurodegeneration. Because of its involvement in the process, the $P2X_7$ receptor might be an interesting and novel target for positron emission tomography (PET) imaging of neuroinflammation, where an ideal PET ligand is still missing.

To date, a variety of $P2X_7$ receptor antagonists have been developed as potential pharmaceuticals, for example, benzoxazinones, (cyano)guanidines and (iso)quinoline carboxamides.[8,9] A-740003, a cyanoguanidine derivative, is a highly selective $P2X_7$ receptor antagonist that displays comparable IC_{50} values for both rat and

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human $P2X_7$ receptors (18 and 40 nM, respectively),[10,11] which make it a good candidate for translation from rodent to human. Therefore, A-740003 is considered to be a promising compound for developing a PET radiotracer for imaging of neuroinflammation. A-740003 is a racemic compound and the affinity of the separate enantiomers may differ.[11] Nevertheless, for initial studies, racemic [^{11}C]A-740003 was synthesised to enable preliminary *in vivo* studies on tracer metabolism and biodistribution in healthy animals.

Experimental

General

Chemicals were obtained from commercial sources and used without further purification. Solvents were purchased from Sigma-Aldrich (Zwijndrecht, the Netherlands) and Biosolve (Valkenswaard, the Netherlands) and used as received unless stated otherwise. Dichloromethane (DCM) and *N,N*-dimethylformamide (DMF) were dried over 3 Å molecular sieves. Tetrahydrofuran (THF) was first distilled from $LiAlH_4$ and then stored on 3 Å molecular sieves. Reactions were performed at room temperature unless stated otherwise. Reactions were monitored by thin layer chromatography on pre-coated silica 60 F254 aluminium plates (Merck, Darmstadt, Germany). Spots were visualised by UV light, $KMnO_4$, anisaldehyde, bromocresol green or 4,4′-tetramethyldiamino-diphenylmethane-Cl_2. Evaporation of solvents was performed under reduced pressure at 40 °C using a rotary evaporator. Flash column chromatography was performed manually on Silica gel 60 Å (Merck, Darmstadt, Germany) or on a Büchi (Flawil, Switzerland) Sepacore system (comprising a C-620 control unit, a C-660 fraction collector, two C-601 gradient pumps and a C-640 UV detector) equipped with Büchi Sepacore pre-packed flash columns. NMR spectroscopy was performed on a Bruker (Billerica, MA, USA) Avance 250 (250.13 MHz for ^{1}H and 62.90 MHz for ^{13}C) or a Bruker Avance 500 (500.23 MHz for ^{1}H and 125.78 MHz for ^{13}C) with chemical shifts (δ) reported in parts per million (ppm) relative to the solvent ($CDCl_3$, ^{1}H 7.26 ppm, ^{13}C 77.16 ppm; CD_3OD, ^{1}H 3.31 ppm, ^{13}C 49.00 ppm; dimethyl sulfoxide (DMSO)-*d6*, ^{1}H 2.50 ppm, ^{13}C 39.52 ppm). Electrospray ionisation-high resolution mass spectrometry (ESI-HRMS) was carried out using a Bruker microTOF-Q instrument in positive ion mode (capillary potential of 4500 V). Analytical isocratic HPLC was performed on a Jasco (Easton, MD, USA) PU-1580 station with a Xbridge C18 5 μm (4.6 × 100 mm) column (Waters, Milford, MA, USA) using acetonitrile (MeCN)/H_2O (30:70, *v/v*) as eluent at a flow rate of 1 mL · min^{-1} (method A), a Jasco UV-2075 Plus UV detector (254 nm) and a NaI radioactivity detector (Raytest, Straubenhardt, Germany). Chromatograms were acquired with Raytest GINA Star software (version 5.01). Semi-preparative isocratic HPLC was performed on a Jasco PU-1587 station with a Reprospher C18-DE 5 μm (50 × 8 mm) column (Dr. Maisch, Ammerbuch-Entringen, Germany) using MeCN/H_2O/diisopropylamine (DIPA), (22:78:0.1, *v/v/v*) as eluent at a flow rate of 3 mL · min^{-1} (method B), a Jasco UV-1575 UV detector (254 nm), a custom-made radioactivity detector and Jasco ChromNAV CFR software (version 1.14.01). Analytical HPLC for metabolite analysis was performed with Dionex (Sunnyvale, CA, USA) UltiMate 3000 HPLC equipment with Chromeleon software (version 6.8) on a Gemini C18 5-μm (10 × 250 mm) column (Phenomenex, Torrance, CA, USA) with gradient and a mixture of MeCN (A) and 0.1% trifluoroacetic acid in water (B) as eluent according to the following scheme (method C): 0 min, 90% B at 0.25 mL · min^{-1}; 0.5 min, 90% B at 4 mL · min^{-1}; 9.0 min, 30% B at 4 mL · min^{-1}; 13.0 min, 30% B at 4 mL · min^{-1}; 14.0 min, 90% B at 4 mL · min^{-1}; and 15.0 min, 90% B at 0.25 mL · min^{-1}. Healthy male Wistar rats were obtained from Harlan Netherlands B.V. (Horst, the Netherlands). All animal experiments were performed according to the National Institute of Health principles of laboratory animal care and Dutch national law ('Wet op de proefdieren', Stb 1985, 336).

Synthesis

*N″-cyano-N-5-quinolinyl guanidine (**2**)*[11]

Water (6 mL) was added to 5-quinolinamine (1; 1.35 g, 9.37 mmol) and 6 M HCl (2 mL) to give a dark red solution. Sodium dicyanamide (1.08 g, 12.1 mmol) was added and the reaction was stirred at 60 °C for 24 h. Another equivalent of both sodium dicyanamide and HCl was added after 24 and 31 h and the reaction was stirred at 60 °C for 16 more hours. The yellow precipitate was collected by filtration. The precipitate was washed twice with cold water (10 mL) and twice with cold Et_2O (10 mL) and finally dried in a vacuum oven for 27 h at 40 °C to obtain *N″*-cyano-*N*-5-quinolinyl guanidine (**2**) in 76% yield (1.77 g, 7.17 mmol).

R_f value, 0.73 (DCM/methanol (MeOH), 9:1); ^{1}H NMR (250.13 MHz, DMSO-*d6*) δ 9.66 (s, 1H, NH), 9.09 (dd, 1H, *J* = 1.4 Hz, 4.5 Hz, CH_{Ar}), 8.72 (d, 1H, *J* = 8.5 Hz, CH_{Ar}), 8.01 (d, 1H, *J* = 8.4 Hz, CH_{Ar}), 7.75–7.83 (m, 3H, CH_{Ar}), 7.37 (s, 2H, NH_2); ^{13}C NMR (125.78 MHz, DMSO-*d6*) δ 161.38 ($C_{guanidine}$), 149.26 (CH_{Ar}), 134.45 (C_{Ar}), 131.60 (CH_{Ar}), 124.78 (CH_{Ar}), 124.27 (CH_{Ar}), 122.00 (CH_{Ar}), 117.68 (CN); ESI-HRMS: calculated for $C_{11}H_9N_5$: 211.09; found 212.09 $[M+H]^+$.

*2-(3-(benzyloxy)-4-methoxyphenyl)acetic acid (**4**)*

Benzyl bromide (150 μL, 1.26 mmol) was added to a solution of 2-(3-hydroxy-4-methoxyphenyl)acetic acid (3; 203 mg, 1.11 mmol) and tetrabutylphosphonium hydroxide (40% wt, 1.5 mL) in THF (2 mL) at 0 °C. The reaction mixture was stirred for 3 h whilst warming to room temperature. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in water (10 mL) and extracted with DCM (3 × 10 mL). The aqueous layer was acidified to pH 1 with concentrated HCl and extracted with DCM (3 × 10 mL). Combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography was performed (hexanes/ethyl acetate (EtOAc) 2:1 + 0.1% acetic acid) to obtain 2-(3-(benzyloxy)-4-methoxyphenyl)acetic acid (**4**) in 73% yield (221 mg, 0.81 mmol).

R_f value, 0.36 (2% MeOH in DCM); ^{1}H NMR (250.13 MHz, $CDCl_3$) δ 7.28–7.45 (m, 5H, CH_{Ph}), 6.83–6.87 (m, 3H, CH_{Ar}), 5.13 (s, 2H, $O\underline{CH_2}Ph$), 3.87 (s, 3H, CH_3), 3.54 (s, 2H, $\underline{CH_2}COOH$); ^{13}C NMR (125.78 MHz, $CDCl_3$) δ 177.03 (COOH), 149.23 ($\underline{C}OCH_2Ph$), 148.35 ($\underline{C}OCH_3$), 137.11 ($C_{Ar-benzyl}$), 128.67 (CH_{Ar-Ph}), 128.01 (CH_{Ar-Ph}), 127.58 (CH_{Ar-Ph}), 125.79 ($\underline{C}CH_2COOH$), 122.33 (CH_{Ar}), 115.44 (CH_{Ar}), 112.05 (CH_{Ar}), 71.23 ($O\underline{CH_2}Ph$), 56.19 (CH_3), 40.51 (CH_2COOH); ESI-HRMS: calculated for $C_{16}H_{16}O_4$: 272.10; found 295.09 $[M+Na]^+$.

*2-(3-(benzyloxy)-4-methoxyphenyl)acetamide (**5**)*

2-(3-(benzyloxy)-4-methoxyphenyl)acetic acid (4; 4.5 g, 16.5 mmol) was dissolved in dry DCM (30 mL). Thionyl chloride (4 mL, 55 mmol) and a drop of DMF were added and the mixture was stirred for 2 h, after which the reaction mixture was concentrated *in vacuo*. The residue was dissolved in dry THF (60 mL), and anhydrous ammonia gas was bubbled through the solution for 5 min. A white precipitate was formed. The reaction mixture was then partitioned in EtOAc (50 mL) and H_2O (30 mL). The aqueous layer was separated and extracted with EtOAc (2 × 30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to obtain 2-(3-(benzyloxy)-4-methoxyphenyl) acetamide (**5**) in 92% yield (4.12 g, 15.2 mmol).

R_f value, 0.24 (2% MeOH in DCM); ^{1}H NMR (250.13 MHz, $CDCl_3$) δ 7.27–7.44 (m, 5H, CH_{Ph}), 6.77–6.88 (m, 3H, CH_{Ar}), 5.57 (s, 1H, NH_2), 5.32 (s, 1H, NH_2), 5.14 (s, 2H, $O\underline{CH_2}Ph$), 3.88 (s, 3H, CH_3), 3.46 (s, 2H, $\underline{CH_2}C(O)NH_2$); ^{13}C NMR (125.78 MHz, $CDCl_3$) δ 173.99 (C=O), 149.27 ($\underline{C}OCH_2Ph$), 148.43 ($\underline{C}OCH_3$), 136.93 ($C_{Ar-benzyl}$), 128.69 (CH_{Ar-Ph}), 128.06 (CH_{Ar-Ph}), 127.54 (CH_{Ar-Ph}), 127.31 ($\underline{C}CH_2C(O)NH_2$), 122.36 ($CH_{Ar}$), 115.47 ($CH_{Ar}$), 112.34 ($CH_{Ar}$), 71.13 ($O\underline{CH_2}Ph$), 56.12 ($CH_3$), 42.91 ($\underline{CH_2}C(O)NH_2$); ESI-HRMS: calculated for $C_{16}H_{17}NO_3$: 271.12; found 272.13 $[M+H]^+$, 294.11 $[M+Na]^+$.

*N-(1-(1H-benzotriazol-1-yl)-2,2-dimethylpropyl)-2-(3-(benzyloxy)-4-methoxyphenyl)acetamide (**6**)*

To a suspension of 2-(3-(benzyloxy)-4-methoxyphenyl)acetamide (5; 4.12 g, 15.2 mmol), 1*H*-benzotriazole (1.81 g, 15.2 mmol) and pivalaldehyde (3.5 mL, 32 mmol) in toluene (50 mL) on $MgSO_4$ (3.0 g, 25 mmol), *p*-toluenesulphonic acid (144 mg, 0.8 mmol) was added. After refluxing overnight, the reaction mixture was cooled at room temperature and DCM (30 mL) was added. $MgSO_4$ was filtered off and the filtrate was

concentrated *in vacuo*. Flash column chromatography was performed (20–50%, EtOAc/hexanes + 0.1% triethylamine) to obtain *N*-(1-(1*H*-benzotriazol-1-yl)-2,2-dimethylpropyl)-2-(3-(benzyloxy)-4-methoxyphenyl) acetamide (**6**) in 74% yield (5.12 g, 11.2 mmol).

R_f value, 0.53 (EtOAc/hexanes, 1:1); ^{1}H NMR (250.13 MHz, $CDCl_3$) δ 8.04 (d, 1H, J = 8.3 Hz, CH_{Ar}), 7.65 (d, 1H, J = 8.3 Hz, CH_{Ar}), 7.27–7.51 (m, 7H, CH_{Ph} + CH_{Ar}), 6.76–6.90 (m, 4H, CH_{Ar} + NH), 6.44 (d, 1H, J = 9.8 Hz, CH), 5.09 (q, 2H, J = 3.0 Hz, 12.2 Hz, $O\underline{CH_2}Ph$), 3.89 (s, 3H, CH_3), 3.49 (q, 2H, J = 8.7 Hz, 16.2 Hz, $\underline{CH_2}C(O)NH_2$), 0.93 (s, 9H, 3x CH_3); ^{13}C NMR (125.78 MHz, $CDCl_3$) δ 171.02 (C=O), 149.24 (C_{Ar}), 148.68 (C_{Ar}), 144.86 (C_{Ar}), 136.82 (C_{Ar}), 133.75 (C_{Ar}), 128.58 (CH_{Ar}), 127.92 (CH_{Ar}), 127.71 (CH_{Ar}), 127.33 (CH_{Ar}), 126.25 (C_{Ar}), 124.06 (CH_{Ar}), 122.21 (CH_{Ar}), 119.75 (CH_{Ar}), 114.85 (CH_{Ar}), 112.26 (CH_{Ar}), 109.95 (CH_{Ar}), 71.04 ($O\underline{CH_2}Ph$), 68.80 ($\underline{CHC}(CH_3)_3$), 56.06 (CH_3), 42.96 (CH_2), 37.04 ($\underline{C}(CH_3)_3$), 25.93 ($C(\underline{CH_3})_3$); ESI-HRMS: calculated for $C_{27}H_{30}N_4O_3$: 458.23; found 459.24 $[M+H]^+$, 481.22 $[M+Na]^+$.

*2-(3-(benzyloxy)-4-methoxyphenyl)-N-(1-(2-cyano-3-(quinolin-5-yl) guanidino)-2,2-dimethylpropyl)acetamide (**7**)*

N-(1-(1*H*-benzotriazol-1-yl)-2,2-dimethylpropyl)-2-(3-(benzyloxy)-4-methoxyphenyl)acetamide (6; 593 mg, 1.29 mmol) and *N*″-cyano-*N*-5-quinolinyl guanidine (2; 282 mg, 1.34 mmol) were dissolved in dry DMF (10 mL) and treated with finely powdered Cs_2CO_3, (1.09 g, 3.34 mmol). After stirring for 25 h, the brown solution was partitioned between EtOAc (20 mL) and H_2O (10 mL), and the aqueous layer was extracted twice with EtOAc (20 mL). The combined organic layers were washed with water (10 mL) once, dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography was performed twice (1–5%, MeOH/DCM) to obtain 2-(3-(benzyloxy)-4-methoxyphenyl)-*N*-(1-(2-cyano-3-(quinolin-5-yl) guanidino)-2,2-dimethylpropyl)acetamide (**7**) as a light orange solid in 47% yield (0.34 g, 0.61 mmol).

R_f value, 0.26 (2% MeOH in DCM); ^{1}H NMR (500.23 MHz, $CDCl_3$) δ 9.74 (s, 1H, NH), 8.94 (dd, 1H, J = 1.3 Hz, 4.1 Hz, CH_{Ar}), 8.24 (d, 1H, J = 8.5 Hz, CH_{Ar}), 8.06 (d, 1H, J = 8.5 Hz, CH_{Ar}), 7.70 (t, 1H, J = 7.9 Hz, CH_{Ar}), 7.53 (d, 1H, J = 7.6 Hz, CH_{Ar}), 7.41–7.44 (m, 3H, CH_{Ar}), 7.33–7.38 (m, 3H, CH_{Ar}), 6.83–6.92 (m, 3H, CH_{Ar}), 6.55 (s, 1H, NH), 5.89 (s, 1H, NH), 5.19 (t, 1H, J = 9.1 Hz, CH), 5.14 (s, 2H, $O\underline{CH_2}Ph$), 3.92 (s, 3H, CH_3), 3.59 (s, 2H, CH_2), 0.96 (s, 9H, 3x CH_3); ^{13}C NMR (125.78 MHz, $CDCl_3$) δ 160.08 ($C_{guanidine}$), 150.60 (CH_{Ar}), 149.48 (C_{Ar}), 148.66 (C_{Ar}), 148.62 (C_{Ar}), 136.58 (C_{Ar}), 130.86 (CH_{Ar}), 128.85 (CH_{Ar}), 128.62 (CH_{Ar}), 128.09 (CH_{Ar}), 127.21 (CH_{Ar}), 125.62 (C_{Ar}), 124.27 (C_{Ar}) 122.18 (CH_{Ar}), 121.37 (CH_{Ar}), 117.22 (CN), 115.01 (CH_{Ar}), 112.29 (CH_{Ar}), 70.99 ($O\underline{CH_2}Ph$), 65.75 ($\underline{CH}(C(CH_3)_3)$), 56.06 ($CH_3$), 42.70 ($CH_2$), 34.94 ($\underline{C}(CH_3)_3$)), 25.48 ($C(\underline{CH_3})_3$); ESI-HRMS: calculated for $C_{32}H_{34}N_6O_3$: 550.27; found 551.28 $[M+H]^+$.

*N-(1-(1H-benzotriazol-1-yl)-2,2-dimethylpropyl)-2-(3-hydroxy-4-methoxyphenyl)acetamide (**9**)*

N-(1-(1*H*-benzotriazol-1-yl)-2,2-dimethylpropyl)-2-(3-(benzyloxy)-4-methoxyphenyl)acetamide (6; 2.4 g, 5.2 mmol) was dissolved in DCM (20 mL). Pd/C (134 mg, 1.3 mmol) was added, and the flask was sealed with a septum and left stirring overnight under hydrogen atmosphere. The reaction mixture was passed over Celite and concentrated *in vacuo*. Flash column chromatography (2–6% MeOH/DCM) was performed to obtain *N*-(1-(1*H*-benzotriazol-1-yl)-2,2-dimethylpropyl)-2-(3-hydroxy-4-methoxyphenyl)acetamide (**9**) in 94% yield (1.8 g, 4.9 mmol).

R_f value, 0.47 (DCM/MeOH, 9:1); ^{1}H NMR (500.23 MHz, $CDCl_3$) δ 8.06 (d, 1H, J = 8.5 Hz, CH_{Ar}), 7.68 (d, 1H, J = 8.5 Hz, CH_{Ar}), 7.35–7.39 (m, 1H, CH_{Ar}) 7.49–7.52 (m, 1H, CH_{Ar}), 6.95 (d, 1H, J = 9.8 Hz, NH), 6.82–6.86 (m, 2H, CH_{Ar}), 6.72 (dd, 1H, J = 1.9 Hz, 7.9 Hz, CH_{Ar}), 6.49 (d, 1H, J = 9.8 Hz, CH), 5.94 (s, 1H, OH), 3.90 (s, 3H, CH_3), 3.54 (q, 2H, J = 16.4 Hz, 27.5 Hz, CH_2), 0.99 (s, 9H, 3x CH_3); ^{13}C NMR (125.78 MHz, $CDCl_3$) δ 171.12 (C = O), 146.20 (C_{Ar}), 146.17 (C_{Ar}), 144.78 (C_{Ar}), 133.75 (C_{Ar}), 127.74 (CH_{Ar}), 126.90 (C_{Ar}), 124.10 (CH_{Ar}), 120.99 (CH_{Ar}), 119.69 (CH_{Ar}), 115.60 (CH_{Ar}), 111.22 (CH_{Ar}), 110.00 (CH_{Ar}), 68.84 ($\underline{CH}(C(CH_3)_3)$), 55.98 ($CH_3$), 42.82 ($CH_2$), 37.10 ($\underline{C}(CH_3)_3$), 25.94 ($C(\underline{CH_3})_3$); ESI-HRMS: calculated for $C_{20}H_{24}N_4O_3$: 368.18; found 369.19 $[M+H]^+$.

*N-(1-(1H-benzotriazol-1-yl)-2,2-dimethylpropyl)-2-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)acetamide (**10**)*

N-(1-(1H-benzo[d][1,2,3]triazol-1-yl)-2,2-dimethylpropyl)-2-(3-hydroxy-4-methoxyphenyl)-acetamide (9; 1.8 g, 4.9 mmol) and *tert*-butyldimethylchlorosilane (TBDMSCl) (1.5 g, 9.9 mmol) were dissolved in dry DCM (70 mL). Imidazole (1.0 g, 14 mmol) was added and the mixture was stirred overnight. After addition of water (70 mL), the mixture was partitioned between water and DCM. The water layer was extracted with DCM (50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (0.5% MeOH in DCM) was performed to obtain *N*-(1-(1*H*-benzotriazol-1-yl)-2,2-dimethylpropyl)-2-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl) acetamide (**10**) in 87% yield (2.0 g, 4.2 mmol).

R_f value, 0.40 (1% MeOH in DCM); ^{1}H NMR (500.23 MHz, $CDCl_3$) δ 8.06 (d, 1H, J = 8.2 Hz, CH_{Ar}), 7.68 (d, 1H, J = 8.2 Hz, CH_{Ar}), 7.49–7.53 (m, 1H, CH_{Ar}), 7.36–7.39 (m, 1H, CH_{Ar}), 6.89 (d, 1H, J = 8.2 Hz, NH), 6.77–6.81 (m, 3H, CH_{Ar}), 6.46 (d, 1H, J = 9.8 Hz, CH), 3.84 (s, 3H, CH_3), 3.53 (q, 2H, J = 16.4 Hz, 54.2 Hz, CH_2), 1.01 (s, 9H, $Si(CH_3)_2C(\underline{CH_3})_3$), 0.96 (s, 9H, $C(CH_3)_3$), 0.17 (d, 6H, J = 4.1 Hz, $Si(\underline{CH_3})_2C(CH_3)_3$); ^{13}C NMR (125.78 MHz, $CDCl_3$) δ 171.04 (C=O), 150.61 (C_{Ar}), 145.59 (C_{Ar}), 144.86 (C_{Ar}), 133.72 (C_{Ar}), 127.66 (CH_{Ar}), 126.30 (C_{Ar}), 124.00 (CH_{Ar}), 122.68 (CH_{Ar}), 122.00 (CH_{Ar}), 119.75 (CH_{Ar}), 112.55 (CH_{Ar}), 109.94 (CH_{Ar}), 68.66 ($\underline{CH}(C(CH_3)_3)$, 55.48 ($CH_3$), 42.82 ($CH_2$), 37.07 ($\underline{C}(CH_3)_3$), 25.91 ($Si(CH_3)_2C(\underline{CH_3})_3$), 25.69 ($C(\underline{CH_3})_3$), 18.45 ($Si(CH_3)_2C(CH_3)_3$), −4.64 ($Si(CH_3)_2C(CH_3)_3$); ESI-HRMS: calculated for $C_{26}H_{38}N_4O_3Si$: 482.27; found 483.28 $[M+H]^+$.

*N-(1-(2-cyano-3-(quinolin-5-yl)guanidino)-2,2-dimethylpropyl)-2-(3-hydroxy-4-methoxyphenyl)acetamide (**8**)*

N-(1-(1*H*-benzotriazol-1-yl)-2,2-dimethylpropyl)-2-(3-((*tert*-butyldimethylsilyl) oxy)-4-methoxyphenyl)acetamide (10; 2.0 g, 4.1 mmol) and *N*″-cyano-*N*-5-quinolinyl guanidine (2; 0.9 g, 4.3 mmol) were dissolved in dry DMF (30 mL) and treated with finely powdered Cs_2CO_3 (3.5 g, 10.7 mmol). After stirring for 25 h, the solution was partitioned between EtOAc (50 mL) and H_2O (10 mL) and the aqueous layer was extracted twice with EtOAc (50 mL). The combined organic layers were washed with brine (2 × 20 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography was performed (1–5%, MeOH/DCM) to obtain *N*-(1-(2-cyano-3-(quinolin-5-yl)guanidino)-2,2-dimethylpropyl)-2-(3-hydroxy-4-methoxyphenyl) acetamide (**8**) as an off-white solid in 29% yield (0.56 g, 1.2 mmol).

R_f value, 0.21 (5% MeOH in DCM); ^{1}H NMR (500.23 MHz, DMSO-*d6*) δ 9.61 (s, 1H, NH), 8.94 (d, 1H, J = 2.8 Hz, CH_{Ar}), 8.89 (s, 1H, OH), 8.27 (d, 1H, J = 8.5 Hz, CH_{Ar}), 8.02 (s, 1H, NH), 7.97 (d, 1H, J = 8.2 Hz, CH_{Ar}), 7.75 (t, 1H, J = 8.2 Hz, CH_{Ar}), 7.55–7.57 (m, 1H, CH_{Ar}), 7.42 (d, 1H, J = 7.3 Hz, CH_{Ar}), 6.83 (d, 1H, J = 8.2 Hz, CH_{Ar}), 6.63–6.72 (m, 3H, CH_{Ar} + NH), 5.52 (t, 1H, J = 9.1 Hz, CH), 3.73 (s, 3H, CH_3), 3.39 (q, 2H, J = 13.8 Hz, CH_2), 0.89 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (125.78 MHz, DMSO-*d6*) δ 170.97 (C=O), 158.71 ($C_{guanidine}$), 150.74 (CH_{Ar}), 148.25 (C_{Ar}), 146.35 (C_{Ar}), 146.23 (C_{Ar}), 133.15 (C_{Ar}), 131.21 (CH_{Ar}), 129.13 (CH_{Ar}), 128.42 (C_{Ar}), 127.85 (CH_{Ar}), 124.69 (C_{Ar}), 124.32 (CH_{Ar}), 121.53 (CH_{Ar}), 119.56 (CH_{Ar}), 116.44 (CH_{Ar}), 116.43 (CN), 112.09 (CH_{Ar}), 64.56 ($\underline{CH}(C(CH_3)_3)$, 55.64 ($CH_3$), 41.52 ($CH_2$), 35.80 ($\underline{C}(CH_3)_3$), 25.26 ($C(\underline{CH_3})_3$); ESI-HRMS: calculated for $C_{25}H_{28}N_6O_3$: 460.22; found 461.21 $[M+H]^+$.

A-740003

Reference compound *N*-(1-{[(cyanoimino)(5-quinolinylamino)methyl] amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide (A-740003) was synthesised with an overall yield of 7% (0.62 g, 1.3 mmol) as described by Perez-Medrano et al.,[11] except for some minor changes: compound **2** was synthesised as described above, ammonia gas was used in the conversion from carboxylic acid to amide and DMF was used as solvent in the coupling of compound **2** to *N*-(1-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide to yield A-740003.

R_f value, 0.48 (DCM/MeOH, 9:1); ^{1}H NMR (500.23 MHz, $CDCl_3$) δ 9.71 (s, 1H, NH), 8.93 (d, 1H, J = 4.1 Hz, CH_{Ar}), 8.22 (d, 1H, J = 8.5 Hz, CH_{Ar}), 8.05 (d, 1H, J = 8.5 Hz, CH_{Ar}), 7.69 (t, 1H, J = 8.5 Hz, CH_{Ar}), 7.50 (d, 1H, J = 7.6 Hz, CH_{Ar}), 7.39–7.42 (m, 1H, CH_{Ar}), 6.79–6.87 (m, 4H, CH_{Ar} + NH), 5.97 (s, 1H, NH), 5.24 (t, 1H, J = 9.2 Hz, CH), 3.89 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 3.52

(s, 2H, CH_2), 1.01 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (125.78 MHz, $CDCl_3$) δ 160.15 ($C_{guanidine}$), 150.66 (CH_{Ar}), 149.51 (C_{Ar}), 148.70 (C_{Ar}), 130.89 (CH_{Ar}), 128.86 (CH_{Ar}), 125.92 (C_{Ar}), 121.61 (CH_{Ar}), 121.41 (CH_{Ar}), 117.33 (CN), 112.29 (CH_{Ar}), 111.66 (CH_{Ar}), 68.74 ($\underline{CH}(C(CH_3)_3)$), 55.96 ($CH_3$), 42.78 ($CH_2$), 35.07 ($\underline{C}(CH_3)_3$), 25.56 ($C(\underline{CH_3})_3$); ESI-HRMS: calculated for $C_{26}H_{30}N_6O_3$: 474.24; found 475.24 $[M+H]^+$.

Radiosynthesis

[^{11}C]A-740003

[^{11}C]CO_2 was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction performed in a 0.5% O_2/N_2 gas mixture using an IBA Cyclone 18/9 cyclotron (IBA, Louvain-la-Neuve, Belgium). [^{11}C]CO_2 was trapped in 100 μL of 0.1 M $LiAlH_4$ in THF. After evaporation of THF by heating the reaction vial to 130 °C under a helium flow, 250 μL 60% HI in water was added. Formed [^{11}C]CH_3I was then distilled to the second reaction vial containing a solution of precursor **8** (0.5 mg, 1.1 μmol) and tetrabutylammonium hydroxide, (1 μL, 1.5 μmol, 40% solution in water) in 250 μL of DMSO. The reaction mixture was heated at 70 °C for 5 min, after which it was diluted with 0.8 mL of water and purified by semi-preparative HPLC (method B). The fraction containing [^{11}C]A-740003 (t_R = 11.4 min) was collected, diluted with 40 mL of water and trapped on a Sep-Pak Plus tC18 cartridge (Waters, Milford, MA, USA), preconditioned with 10 mL of ethanol and 10 mL of water. The cartridge was washed with 20 mL of water and subsequently [^{11}C]A-740003 was eluted with 1 mL of EtOH and diluted with 5 mL of 7.09 mM NaH_2PO_4 in saline. [^{11}C]A-740003 was obtained in a decay-corrected radiochemical yield of 26–59% (n = 2) from end of bombardment with a radiochemical purity >97%, a specific activity (SA) of 41 ± 22 GBq/μmol (n = 3) and an overall synthesis time of approximately 50 min. The identity of the product was confirmed with analytical HPLC by coinjection of the product and non-labelled A-740003 (method A, t_R = 7.4 min).

Biodistribution

Healthy male Wistar rats (210–275 g) were injected with approximately 50 MBq (at start of experiment) [^{11}C]A-740003 in the tail vein under isoflurane anaesthesia. Rats were sacrificed and dissected at 5, 15, 30 and 60 min post-injection (n = 4 for 5 and 30 min and n = 3 for 15 and 60 min). Blood, heart, lungs, liver, kidneys, olfactory bulbs, hippocampus, striatum, cerebral cortex, cerebellum and the rest of the brain were collected, weighed and counted for radioactivity in a Wallac Universal Gamma Counter 1282 (PerkinElmer, Waltham, MA, USA). Biodistribution data were expressed as percentage injected dose per gram tissue (%ID/g).

Metabolite analysis

Healthy male Wistar rats (230–250 g) were injected with approximately 100 MBq (at start of experiment) [^{11}C]A-740003 in the tail vein under isoflurane anaesthesia. Rats were sacrificed at 15 and 45 min (n = 3 per time point). Blood samples were obtained, and the brain was removed. Blood samples were collected in heparin tubes (BD Vacutainer NH 119 I.U., 7 mL, BD Vacitainer Systems, Plymouth, UK) and centrifuged at 4000 rpm for 5 min at 4 °C (Hettich Universal 32, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). The brain was put in a falcon tube containing saline (5 mL) and homogenised with a disperser (IKA T18 B Ultra-Turrax, IKA®-Werke GmbH & Co. KG, Staufen, Germany) before centrifugation (5 min, 4000 rpm, 20 °C, Hettich Universal 32). Plasma and brain supernatant were separated from blood cells and brain precipitate, respectively. Plasma and brain supernatant were loaded onto a Sep-Pak tC18 cartridge, followed by washing with 10 mL of water to obtain the polar fraction. The non-polar fraction was then eluted with 1.5 mL of MeOH and 1.5 mL of water. All separate fractions were counted for radioactivity in a Wizard Gamma Counter 1470 or 2480 (Wallac/PerkinElmer, Waltham, MA, USA) and the non-polar fraction was further analysed by HPLC using method C.

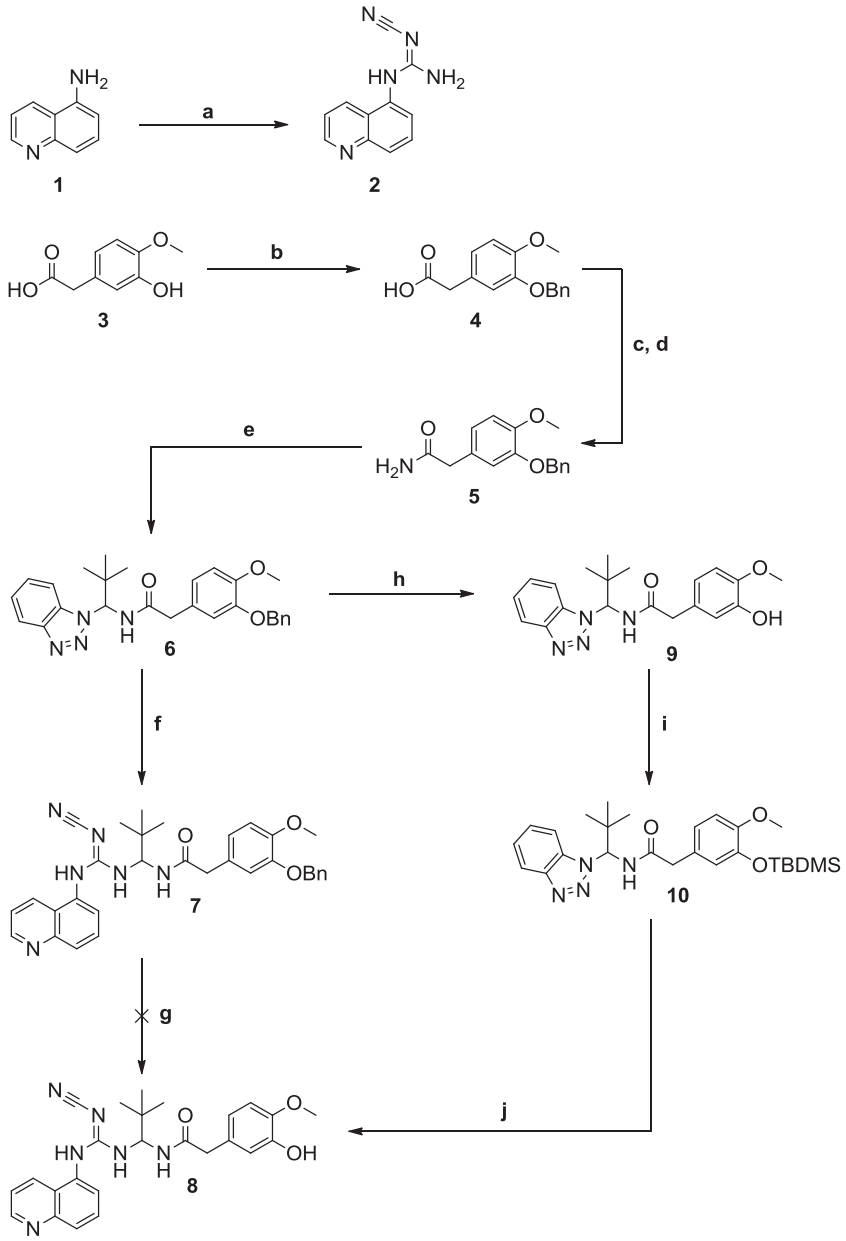
Results and discussion

Synthesis

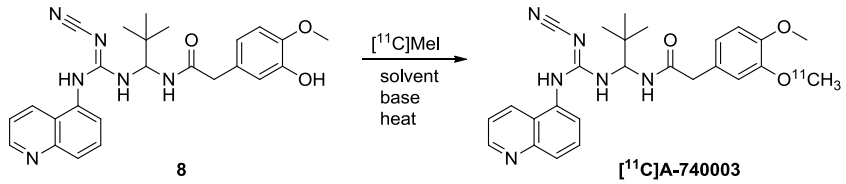
Reference compound A-740003 was synthesised according to a procedure previously described by Perez-Medrano et al.[11] The synthesis route towards precursor **8**, depicted in Scheme 1, was adapted from this procedure. In order to be able to incorporate a [^{11}C]methyl group at the phenolic hydroxyl of precursor **8**, use of a protective group at this position that would withstand all reaction conditions throughout the synthesis and could be removed at the last step was required. Allyl and benzyl protecting groups were selected for this purpose.[12] The latter was favoured on the basis of test reactions in which deprotection of benzyl-protected acetamide **5** or allyl protected acetamide (structure not shown) was performed. The synthesis of benzyl-protected precursor **7** was started with reaction of 5-aminoquinoline **1** with sodium dicyanamide in water in the presence of HCl resulting in *N*″-cyano-*N*-5-quinolinyl guanidine **2** in 76% yield. The phenolic hydroxyl group of 2-(3-hydroxy-4-methoxyphenyl)acetic acid **3** was protected with benzyl bromide in the presence of tetrabutylphosphonium hydroxide resulting in carboxylic acid **4** in 73% yield.[13] Carboxylic acid **4** was then converted to acetamide **5** in 92% yield by reaction with thionyl chloride followed by treatment of the acid chloride intermediate with ammonia. Reaction of acetamide **5** with pivalaldehyde and benzotriazole yielded compound **6** in 74%. Subsequently, compound **6** was coupled to *N*″-cyano-*N*-5-quinolinyl guanidine **2** in the presence of Cs_2CO_3 to obtain the benzyl-protected precursor **7** in a yield of 47%. Unfortunately, despite the use of several deprotection methods, precursor **8** could not be obtained. The following deprotection conditions were tested: Pd/C with hydrogen, trifluoroacetic acid, titanium trichloride and magnesium, boron trifluoride etherate and sodium iodide and aluminium chloride.[14–16] In all conditions tested, the formation of many different unidentified products as well as reduction of benzyl protected precursor **7** at undesired positions was observed by mass spectrometry. Therefore, an alternative synthesis route towards precursor **8** was developed, starting with benzyl deprotection of **6** with hydrogen and Pd/C. Subsequently, the phenolic hydroxyl group of compound **9** was protected with TBDMSCl, in the presence of imidazole in a yield of 77%. TBDMS was selected for its mild and selective cleavage. TBDMS-protected compound **10** was then coupled to *N*″-cyano-*N*-5-quinolinyl guanidine **2**, during which unforeseen but complete TBDMS deprotection occurred, resulting in precursor **8** in 29% yield. TBDMS deprotection was probably because of the presence of trace amounts of water.[17,18] All other yields obtained were in accordance with those reported in literature.

Radiosynthesis

In order to obtain [^{11}C]A-740003, the phenolic hydroxyl group of precursor **8** was subjected to carbon-11 methylation by reaction with [^{11}C]methyl iodide in the presence of a base (Scheme 2). Although radiolabelling of phenolic hydroxyl groups with [^{11}C]methyl iodide is often performed in the presence of sodium hydroxide,[19–23] this was not successful for precursor **8** using either DMF or DMSO as a solvent (Table 1). However, using the milder base tetrabutylammonium hydroxide, conversion of [^{11}C]methyl iodide to [^{11}C]A-740003 was on average 87% (n = 4, determined as percentage of the total activity of the crude reaction mixture observed on analytical HPLC). During



Scheme 1. Synthesis route towards precursor **8**. Reagents and conditions: a) $NaN(CN)_2$, HCl, H_2O, 60 °C, 47 h, 76%; b) *n*-Bu_4POH, BnBr, THF, 3 h, 73%; c) $SOCl_2$, DMF, DCM, 1 h; d) NH_3, THF, 5 min, 92% (over two steps); e) 1*H*-benzotriazole, pivalaldehyde, *p*TsOH, $MgSO_4$, toluene, reflux, 16 h, 74%; f) **2**, Cs_2CO_3, DMF, 25 h, 47%; g) several benzyl deprotection methods; h) H_2, Pd/C, DCM, 16 h, 94%; i) TBDMSCl, imidazole, DCM, 16 h, 87%; j) **2**, Cs_2CO_3, DMF, 25 h, 29%.



Scheme 2. Radiosynthesis of [^{11}C]A-740003.

purification of the radiolabelled product by means of semi-preparative HPLC, some difficulties (e.g. no retention of radiolabelled product) were encountered that could be solved by dilution of the reaction mixture with 0.8 mL water prior to semi-preparative HPLC purification. Subsequently, the product was formulated in 6 mL of 17% EtOH in 7.09 mM NaH_2PO_4 in saline to obtain [^{11}C]A-740003 (2.9–5.4 GBq) with sufficient radiochemical purity (>97%) and SA (41 ± 22 GBq/μmol) to be evaluated *in vivo*. Formulated [^{11}C]A-740003 was determined to be stable until injection by analytical HPLC.

Table 1. Optimisation radiolabelling [^{11}C]A-740003

Solvent (250 μL)	Base	T (°C)	Conversion on analytical HPLC	RCY (d.c.)	SA (GBq/μmol)
DMF	NaOH (5 μmol)	80	<1% ($n = 1$)	n.d.	n.d.
DMSO	NaOH (5 μmol)	70	<1% ($n = 2$)	n.d.	n.d.
DMSO	TBAOH (1.5 μmol)	70	87 ± 6% ($n = 4$)[a]	26–59% ($n = 2$)[b]	41 ± 22 ($n = 3$)[b]

Precursor concentration = 4 mM; reaction time = 5 min.
n.d., not determined.
[a]Conversion was not determined in tracer productions for animal experiments.
[b]Because of optimisation of purification, only two syntheses were identically performed technically.

Metabolite analysis

To assess *in vivo* stability of [^{11}C]A-740003, healthy male Wistar rats were injected with around 100 MBq of the formulated radiolabelled compound. The amount of intact [^{11}C]A-740003 in plasma was determined to be 59% and 31% at 15 and 45 min after injection, respectively (Table 2). Two unidentified non-polar metabolites were observed on HPLC as can be seen in Figure 1.

In brain supernatant, the measured amount of non-polar radioactive compound was 8% and 3% at 15 and 45 min after injection, respectively. The percentage of intact tracer in the non-polar fraction could not be identified with HPLC, because the amount of activity in the brain was too low. Furthermore, the amount of polar metabolites was substantially higher in the brain than in plasma, which is highly unlikely. This might be explained by the low brain uptake of the parent tracer (as seen in the biodistribution study, Figure 2), causing a distorted view due to radioactive species remaining in the blood vessels of the brain. Radioactivity in the brain could therefore essentially be allocated to blood born polar metabolites.

Biodistribution

In the biodistribution study, around 50 MBq of formulated [^{11}C]A-740003 was injected in healthy male Wistar rats. [^{11}C]A-740003 showed clearance via kidneys and liver (Figure 2), although excretion via intestines could still be possible. At all time points, tracer uptake observed in brain regions was low and even close to background measurements.

Table 2. Metabolite analysis of [^{11}C]A-740003 in plasma and brain supernatant

	Plasma		Brain	
	15 min (%)	45 min (%)	15 min (%)	45 min (%)
Non-polar fraction	70 ± 6	43 ± 2	8 ± 1%	3 ± 1%
Intact tracer	59 ± 7	31 ± 2	n.d.	n.d.
Non-polar metabolites	11 ± 1	12 ± 1	n.d.	n.d.
Polar metabolites	30 ± 6	57 ± 2	92 ± 1%	97 ± 2%

n.d., not determined.
$n = 3$ for all time points.

High variability in SA of [^{11}C]A-740003 between experiments was observed (5–81 GBq/μmol, corresponding with injection of 0.3–4.7 μg of carrier). With lower SA, more carrier was injected, which may have affected pharmacokinetics. Overall, higher values would be expected with lower SA due to longer circulation in the blood. To correct for the variable SA, organ-to-blood ratios were calculated as depicted for the brain in Figure 3. These data suggest that there is a slight increase in tracer uptake over time or that clearance of radioactivity is faster in blood than in brain due to radioactive metabolites present in blood.

The low brain uptake indicates that either the tracer does not enter the brain through the blood–brain barrier or that it is not retained in the brain due to the low expression level of $P2X_7$ receptors in healthy subjects. $P2X_7$ receptor expression, however, was shown to be widespread in rat brain by means of autoradiography, with moderate-high tritiated ligand binding in several brain regions, including cerebral cortex, hippocampus and olfactory nucleus, and a maximum number of receptor binding sites (B_{max}) in brain of 112 fmol · mg^{-1} has been reported.[24] For comparison, B_{max} values for the metabotropic glutamate 5 (mGlu5) receptor in rat brain are twofold to eightfold higher and range from 231 to 870 fmol · mg^{-1}.[25–27] In recent years, successful radiopharmaceuticals for the mGlu5 receptor have been published.[26,28] Uptake of these radiopharmaceuticals in whole rat brain at 30 min post-injection was 0.074, 0.057 and 0.128%ID/cc for [^{11}C]M-PEPy, [^{11}C]M-MPEP and [^{11}C]MPEP, respectively.[28] For [^{11}C]ABP688, uptake was 0.19 and 0.22%ID/g in hippocampus and striatum, respectively.[26] The observed lower brain uptake of [^{11}C]A-740003 (about 0.03% ID/g at 30 min post-injection, Figure 2) might therefore be explained by the lower B_{max} value for the $P2X_7$ receptor compared with the mGlu5 receptor. Nevertheless, the brain region-to-blood ratios suggest a slow but steady increase of tracer over time.

In summary, [^{11}C]A-740003 was successfully synthesised and, in healthy male Wistar rats, low brain uptake and a moderate metabolic rate were observed. To the best of our knowledge, [^{11}C]A-740003 is the first potential PET tracer for the $P2X_7$ receptor that has been synthesised and evaluated *in vivo*. Further studies are needed to assess whether [^{11}C]A-740003 shows higher brain uptake in animal models of neuroinflammation with increased $P2X_7$ receptor expression, thus to evaluate its potential as a novel PET tracer.

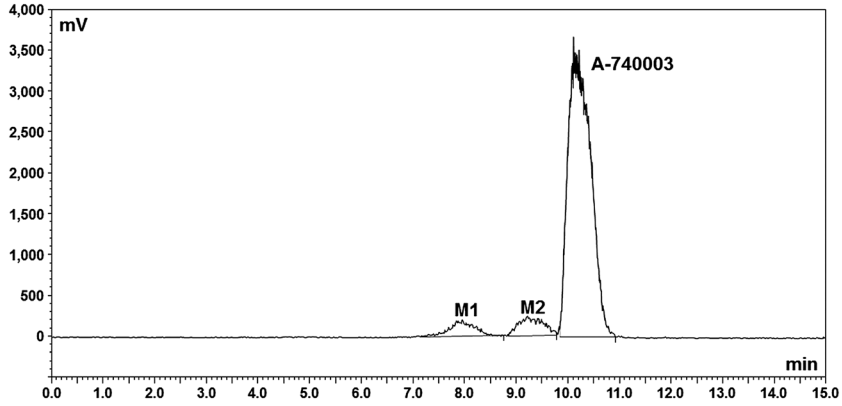


Figure 1. Radiochromatogram of the non-polar fraction of plasma after 15 min. Intact [^{11}C]A-740003 has a retention time of 10.1 min.

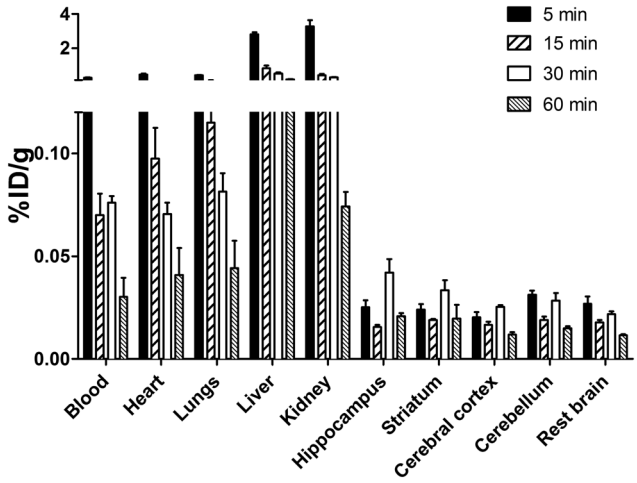


Figure 2. Biodistribution following intravenous injection of about 50 MBq of [^{11}C] A-740003 in healthy male Wistar rats ($n = 3$ for 5 and 60 min; $n = 4$ for 15 and 30 min). Data are expressed as percentage injected dose per gram tissue (%ID/g).

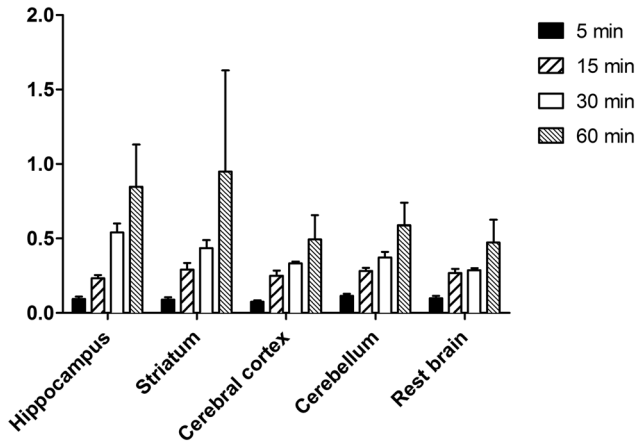


Figure 3. Brain region-to-blood ratio of [^{11}C]A-740003 (n = 3 for 5 and 60 min; n = 4 for 15 and 30 min.

Conclusion

The $P2X_7$ receptor antagonist [^{11}C]A-740003 was successfully synthesised but showed little uptake in brain in healthy male Wistar rats.

Acknowledgements

The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. HEALTH-F2-2011-278850 (INMiND). J. Verbeek is acknowledged for his assistance during [^{11}C]methylation experiments.

Conflict of interest

The authors did not report any conflict of interest.

References

[1] C. K. Glass, K. Saijo, B. Winner, M. C. Marchetto, F. H. Gage, *Cell* **2010**, *140*, 918.
[2] M. Monif, G. Burnstock, D. A. Williams, *Int. J. Biochem. Cell Biol.* **2010**, *42*, 1753.
[3] T. Takenouchi, K. Sekiyama, A. Sekigawa, M. Fujita, M. Waragai, S. Sugama, Y. Iwamaru, H. Kitani, M. Hashimoto, *Arch. Immunol. Ther. Exp.* **2010**, *58*, 91.
[4] Y. Yiangou, P. Facer, P. Durrenberger, I. P. Chessell, A. Naylor, C. Bountra, R. R. Banati, P. Anand, *BMC Neurol.* **2006**, *6*, 12.
[5] A. H. Jacobs, B. Tavitian, *J. Cereb. Blood Flow Metab.* **2012**, *32*, 1393.
[6] K. Inoue, M. Tsuda, *Exp. Neurol.* **2012**, *234*, 293.
[7] A. Bhattacharya, R. A. Neff, A. D. Wickenden, *Curr. Pharm. Biotechnol.* **2011**, *12*, 1698.
[8] S. D. Guile, L. Alcaraz, T. N. Birkinshaw, K. C. Bowers, M. R. Ebden, M. Furber, M. J. Stocks, *J. Med. Chem.* **2009**, *52*, 3123.
[9] H. Gunosewoyo, M. Kassiou, *Expert Opin. Ther. Pat.* **2010**, *20*, 625.
[10] P. Honore, D. Donnelly-Roberts, M. T. Namovic, G. Hsieh, C. Z. Zhu, J. P. Mikusa, G. Hernandez, C. Zhong, D. M. Gauvin, P. Chandran, R. Harris, A. Perez Medrano, W. Carroll, K. Marsh, J. P. Sullivan, C. R. Faltynek, M. F. Jarvis, *J. Pharmacol. Exp. Ther.* **2006**, *319*, 1376.
[11] A. Perez-Medrano, D. L. Donnelly-Roberts, P. Honore, G. C. Hsieh, M. T. Namovic, S. Peddi, Q. Shuai, Y. Wang, C. R. Faltynek, M. F. Jarvis, W. A. Carroll, *J. Med. Chem.* **2009**, *52*, 3366.
[12] T. W. Greene, P. G. M. Wuts, Greene's Protective Groups in Organic Synthesis, 4th ed., John Wiley & Sons Inc., Hoboken, New Jersey, **2007**.
[13] P. Liu, L. Huang, M. M. Faul, *Tetrahedron Lett.* **2007**, *48*, 7380.
[14] S. M. Kadam, S. K. Nayak, A. Banerji, *Tetrahedron Lett.* **1992**, *33*, 5129.
[15] C. Börger, H.-J. Knölker, *Tetrahedron* **2012**, *68*, 6727.
[16] D. Oohira, K. Otaka, WO 2005/063694, July 14, **2005**.
[17] L. Zhang, E. Meggers, *J. Am. Chem. Soc.* **2005**, *127*, 74.
[18] X. Weng, L. Ren, L. Weng, J. Huang, S. Zhu, X. Zhou, L. Weng, *Angew. Chem. Int. Ed.* **2007**, *46*, 8020.
[19] S. Celen, J. Cleynhens, C. Deroose, T. de Groot, A. Ibrahimi, R. Gijsbers, Z. Debyser, L. Mortelmans, A. Verbruggen, G. Bormans, *Bioorg. Med. Chem.* **2009**, *17*, 5117.

[20] A. van Waarde, N. K. Ramakrishnan, A. A. Rybczynska, P. H. Elsinga, F. Berardi, J. R. de Jong, C. Kwizera, R. Perrone, M. Cantore, J. W. A. Sijbesma, R. A. Dierckx, N. A. Colabufo, *J. Med. Chem.* **2009**, *52*, 4524.
[21] K. K. Solingapuram Sai, K.-E. Kil, Z. Tu, W. Chu, B. N. Finck, J. M. Rothfuss, K. I. Shoghi, M. J. Welch, R. J. Gropler, R. H. Mach, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6233.
[22] J.-Q. Wang, Z. Zhang, D. Kuruppu, A.-L. Brownell, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1958.
[23] J. Caballero, C. Muñoz, J. H. Alzate-Morales, S. Cunha, L. Gano, R. Bergmann, J. Steinbach, T. Kniess, *Eur. J. Med. Chem.* **2012**, *58*, 272.
[24] S. L. Able, R. L. Fish, H. Bye, L. Booth, Y. R. Logan, C. Nathaniel, P. Hayter, S. D. Katugampola, *Br. J. Pharmacol.* **2011**, *162*, 405.
[25] E. Hinoi, K. Ogita, Y. Takeuchi, H. Ohashi, T. Maruyama, Y. Yoneda, *Neurochem. Int.* **2001**, *38*, 277.
[26] S. M. Ametamey, L. J. Kessler, M. Honer, M. T. Wyss, A. Buck, S. Hintermann, Y. P. Auberson, F. Gasparini, P. A. Schubiger, *J. Nucl. Med.* **2006**, *47*, 698.
[27] S. Hintermann, I. Vranesic, H. Allgeier, A. Brülisauer, D. Hoyer, M. Lemaire, T. Moenius, S. Urwyler, S. Whitebread, F. Gasparini, Y. P. Auberson, *Bioorg. Med. Chem.* **2007**, *15*, 903.
[28] M. Yu, W. Tueckmantel, X. Wang, A. Zhu, A. P. Kozikowski, A.-L. Brownell, *Nucl. Med. Biol.* **2005**, *32*, 631.