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(71) Applicant *(for all designated States except US)*: **GE HEALTHCARE LIMITED** [GB/GB]; Amersham Place, Little Chalfont Buckinghamshire HP7 9NA (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **JACKSON, Alexander** [GB/GB]; GE Healthcare Limited, The Grove Centre, White Lion Road, Amersham Buckinghamshire HP7 9LL (GB). **MORISSON-IVESON, Veronique** [FR/GB]; GE Healthcare Limited, The Grove Centre, White Lion Road, Amersham Buckinghamshire HP7 9LL (GB).

(74) Agent: **BRYAN, Ian Bennett**; GE Healthcare Limited, Pollards Wood, Nightingales Lane, Chalfont St Giles Buckinghamshire HP8 4SP (GB).

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(54) Title: IMAGING THE CENTRAL NERVOUS SYSTEM WITH PURINERGIC P2X7 RECEPTOR BINDING AGENTS

(57) Abstract: The present invention provides novel compounds which may be used as *in vivo* imaging agents. The compounds of the invention are useful in a method to image the expression of P2X7 receptors in a subject, as a means to facilitate the diagnosis of a range of disease states.

IMAGING THE CENTRAL NERVOUS SYSTEM WITH PURINERGIC P2X7 RECEPTOR BINDING AGENTS

Technical Field of the Invention

The present invention relates to the field of purinergic P2 receptors. More particularly, the present invention relates to novel purinergic $P2X_7$ receptor *in vivo* imaging agents, their production and intermediates thereof. In further detail, the present invention relates to the use of the *in vivo* imaging agents of the invention in methods to provide information useful in the diagnosis of disease states in which $P2X_7$ receptor expression is implicated.

Description of Related Art

The $P2X_7$ receptor is a cation-selective ion channel directly gated by extracellular ATP (the only known physiological ligand) and a few pharmacological ATP analogues (North 2002 Physiol. Rev. 82:1013-1067). The release of ATP from damaged cells and the subsequent activation of purinergic $P2X_7$ receptors located on hematopoietic cells (such as microglia, macrophages and lymphocytes) is crucial to the inflammatory cascade (Ferrari D *et al* 2006 J. Immunol. 176:3877-83). The cation movement associated with the opening of the plasma membrane $P2X_7$ channel is necessary for the maturation and release of the main pro-inflammatory cytokine, interleukin-1ß (IL-1ß). While the expression of $P2X_7$ is low in normal tissue, during inflammation (whether central or peripheral) there is a large increase in $P2X_7$ reactivity on cells in the surrounding area.

In the central nervous system (CNS), increases in $P2X_7$ have been characterised following the experimental inducement of stroke (Franke *et al* 2004 J. Neuropathol. Exp. Neurol. 63:686-99); multiple sclerosis (MS) (Yiangou *et al* 2006 BMC. Neurol. 6:12); amyotrophic lateral sclerosis (ALS) (Yiangou *et al* 2006 *supra*); epilepsy (Rappold *et al* 2006 Brain Res. 1089:171-8); and, in a transgenic, amyloidic Alzheimer's disease mouse (Parvathenani *et al* 2003 J. Biol. Chem. 278:13309-17). In the periphery, $P2X_7$ receptor upregulation has been shown to accompany neuropathic pain (Chessell *et al* 2005 Pain 114:386-96); polycystic kidney disease (Franco-Martinez *et al* 2006 Clin. Exp. Immunol. 146:253-61); and, tuberculosis (Hillman *et al* 2005

Nephron. Exp. Nephrol. 101:e24-30). $P2X_7$ upregulation has also been shown in a variety of cancers, e.g. cervical, uterine, prostate, breast and skin cancers and leukaemias, both in experimental models and in patients (Feng *et al* 2006 J. Biol. Chem. 281:17228-37; Greig *et al* 2003 J. Invest. Dermatol. 121:315-327; Slater *et al* 2004 Histopathology 44:206-215 Slater *et al* 2004 Breast Cancer Res. Treat. 83:1-10; Zhang *et al* 2004 Leuk. Res 28:1313-1322; Li *et al* 2006 Cancer Epidemiol. Biomarkers Prev. 15:1906-13).

A number of compound classes have been synthesised from different structural backbones to generate therapeutic $P2X_7$ antagonists. A review of agonists and antagonists acting at the $P2X_7$ receptor has been published by Baraldi *et al* (2004 Curr. Topics Med. Chem. 4:1707-17). The compounds disclosed therein are discussed as being potentially useful therapeutic agents. Small molecule $P2X_7$ binding compounds have also been disclosed in relation to *in vivo* imaging applications. WO 2007/141267 provides pyrazole derivatives that are $P2X_7$ antagonists for the treatment of pain, inflammation and neurodegeneration. Isotopically-labelled versions of the compounds are taught to be useful for *in vivo* imaging by single-photon emission tomography (SPECT) or PET. WO 2007/109154 and WO 2007/109192 disclose bicycloheteroaryl compounds as $P2X_7$ modulators. Isotopic variants of these comprising ^{11}C, ^{18}F, ^{15}O or ^{13}N are taught to be useful in PET studies of substrate receptor occupancy. WO 2008/064432 discloses polycyclic compounds for the diagnosis, treatment or monitoring of disorders in which the $P2X_7$ receptor is implicated. Compounds of WO 2008/064432 that were tested in a $P2X_7$ receptor functional assay demonstrated that the compounds were antagonists of the $P2X_7$ receptor. The compounds of WO 2008/064432 may be radiolabelled with an isotope suitable for *in vivo* imaging, e.g. by SPECT or PET.

There is scope for an alternative *in vivo* imaging agent suitable for imaging the $P2X_7$ receptor to facilitate the diagnosis of the broad range of disease states associated with the $P2X_7$ receptor, in particular those of the central nervous system (CNS).

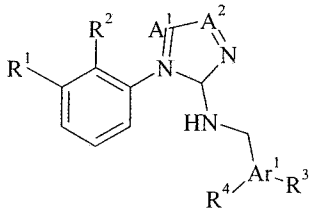
Summary of the Invention

The present invention provides novel compounds which may be used as *in vivo* imaging

agents. The *in vivo* imaging agents of the invention are particularly useful in a method to image the expression of $P2X_7$ receptors in the CNS of a subject, as a means to facilitate the diagnosis of a range of disease states.

Detailed Description of the Invention

In one aspect, the present invention provides an *in vivo* imaging agent suitable for *in vivo* imaging the central nervous system (CNS) of a subject, wherein said *in vivo* imaging agent comprises a compound of Formula I, or a salt or solvate thereof, wherein Formula I is defined as follows:



(I)

wherein:

R^1 and R^2 are independently selected from hydrogen, halo, hydroxyl, C_{1-3} alkyl, C_{1-3} fluoroalkyl, and C_{1-3} hydroxyalkyl;

R^3 and R^4 are independently selected from hydrogen, halo, hydroxyl, C_{1-3} alkyl, C_{1-3} fluoroalkyl, C_{1-3} hydroxyalkyl, C_{1-3} alkyloxy, C_{1-3} fluoroalkyloxy, C_{1-3} alkylthio, C_{1-3} fluoroalkylthio and C_{1-6} cycloalkyl;

one of A^1 and A^2 is N and the other is CH;

Ar^1 is a C_{5-12} aryl group optionally comprising 1-3 heteroatoms selected from nitrogen, oxygen and sulfur; and,

wherein any one of R^1, R^2, R^3 and R^4 as defined comprises an *in vivo* imaging moiety which is a gamma-emitting radioactive halogen or a positron-emitting radioactive non-metal.

The term "*in vivo* imaging agent" refers to a compound which can be used to detect a particular physiology or pathophysiology in a living subject by means of its administration

to said subject and subsequent detection within said subject, wherein detection is carried out external to said subject.

In order to be "suitable for *in vivo* imaging of the central nervous system (CNS)" an *in vivo* imaging agent needs to be able to cross the blood-brain barrier (BBB). The "CNS" is that part of the nervous system of a subject comprising the brain and spinal cord that is covered by the meninges. The generally accepted biophysical/physicochemical models of BBB penetration have as their primary determinants for passive transport: the solute's lipophilicity; hydrogen-bond desolvation potential; pKa/charge; and, molecular size. Typically, a suitable lipophilicity value for a compound to penetrate the BBB would be LogP in the range 1.0-4.5, preferably 2.0-3.5.

The "subject" of the invention is preferably a mammal, most preferably an intact mammalian body *in vivo*. In an especially preferred embodiment, the subject of the invention is a human.

In the term "salt or solvate thereof", a suitable salt may be selected from (i) physiologically acceptable acid addition salts such as those derived from mineral acids, for example hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulphuric acids, and those derived from organic acids, for example tartaric, trifluoroacetic, citric, malic, lactic, fumaric, benzoic, glycollic, gluconic, succinic, methanesulphonic, and para-toluenesulphonic acids; and (ii) physiologically acceptable base salts such as ammonium salts, alkali metal salts (for example those of sodium and potassium), alkaline earth metal salts (for example those of calcium and magnesium), salts with organic bases such as triethanolamine, N-methyl-D-glucamine, piperidine, pyridine, piperazine, and morpholine, and salts with amino acids such as arginine and lysine. A suitable solvate may be selected from those formed with ethanol, water, saline, physiological buffer and glycol.

When a substituent "comprises an *in vivo* imaging moiety" said substituent either is an *in vivo* imaging moiety, or said substituent is a chemical group that includes an *in vivo* imaging moiety, wherein in both cases said *in vivo* imaging moiety is either a gamma-emitting radioactive halogen or a positron-emitting radioactive non-metal. Such a radioactive isotope is present in the *in vivo* imaging agent of the invention at a level

significantly above the natural abundance level of said radioactive isotope. Such elevated or enriched levels of radioactive isotope are suitably at least 5 times, preferably at least 10 times, most preferably at least 20 times; and ideally either at least 50 times the natural abundance level of the radioactive isotope in question, or present at a level where the level of enrichment of the radioactive isotope in question is 90 to 100%. Examples of chemical groups that comprise an *in vivo* imaging moiety suitable for the present invention include iodophenyl groups with elevated levels of ^{123}I, CH_3 groups with elevated levels of ^{11}C, and fluoroalkyl groups with elevated levels of ^{18}F, such that the imaging moiety is the isotopically labelled ^{11}C or ^{18}F atom within the chemical structure. More detailed discussion of how these and other suitable functional groups are incorporated into the *in vivo* imaging agents of the invention is given later on in this description.

An "*in vivo* imaging moiety" allows the compound of the invention to be detected using a suitable imaging modality following its administration to a mammalian body *in vivo*. Suitable imaging modalities of the present invention include positron-emission tomography (PET) and single-photon emission tomography (SPECT).

When the *in vivo* imaging moiety is a "gamma-emitting radioactive halogen", the radiohalogen is suitably chosen from ^{123}I, ^{131}I or ^{77}Br. ^{125}I is specifically excluded as it is not suitable for use in *in vivo* imaging. A preferred gamma-emitting radioactive halogen for *in vivo* imaging is ^{123}I.

When the imaging moiety is a "positron-emitting radioactive non-metal", suitable such positron emitters include: ^{11}C, ^{17}F, ^{18}F, ^{75}Br, ^{76}Br or ^{124}I. Preferred positron-emitting radioactive non-metals are ^{11}C, ^{18}F and ^{124}I, especially ^{11}C and ^{18}F, most especially ^{18}F.

The term "halo" means a substituent selected from fluorine, chlorine, bromine or iodine. "Haloalkyl", "haloacyl", "haloalkoxy" and "haloaryl" are alkyl, acyl, alkoxy and aryl groups, respectively, as defined herein, substituted with one or more halo groups. "Fluoroalkyl", "fluoroalkoxy" and "fluoroalkylthio" are alkyl, alkoxy and alkylthio groups, respectively, as defined herein, substituted with one or more fluoro groups.

Unless otherwise specified, the term "alkyl" alone or in combination, means a straight-chain or branched-chain alkyl radical containing between 1-6 carbon atoms, and

preferably between 1 to 3 carbon atoms. Examples of such radicals include, but are not limited to, methyl, ethyl, n-propyl, and isopropyl.

"Hydroxyl" is the group –OH. The term "hydroxyalkyl" represents an alkyl group as defined herein substituted with one or more hydroxyl groups. Preferably a hydroxyalkyl group is of the structure $-(CH_2)_n-OH$ wherein n is 1-6.

Unless otherwise specified, the term "alkoxy", alone or in combination, means an alkyl as defined above which includes an ether radical in the chain (i.e. the group -O-). Examples of suitable alkyl ether radicals include, but are not limited to, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, iso-butoxy, sec-butoxy, tert-butoxy.

The term "thio" means the group -SH. The terms "alkylthio" and "fluoroalkylthio" represent alkyl and fluoroalkyl groups, respectively, as defined herein substituted with one or more thiol groups.

The term "cycloalkyl" refers to an alkyl as defined herein wherein the ends of the chain are joined to form a cyclic structure.

The term "aryl" refers to aromatic rings or fused aromatic ring systems having 5 to 12 carbon atoms, preferably 5 to 6 carbon atoms, in the ring system, e.g. phenyl or naphthyl. A "heteroatom" is an atom selected from nitrogen, oxygen and sulfur that takes the place of one of the carbon atoms of the aromatic ring. An aryl group comprising one or more heteroatoms is usually termed a "heteroaryl".

Preferably, R^1 and R^2 are independently selected from hydrogen, halo, and hydroxyl.

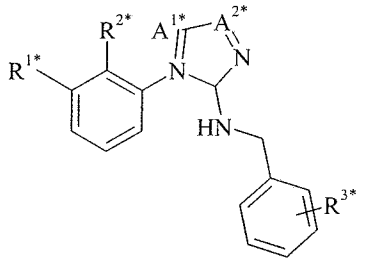
Preferably, R^3 and R^4 are independently selected from hydrogen, hydroxyl, halo, and C_{1-3} fluoroalkoxy.

Preferably, A^1 is N and A^2 is CH.

Preferably, Ar^1 is a C_{5-6} aryl group optionally comprising 1 heteroatom selected from nitrogen, oxygen and sulfur.

In a preferred embodiment, one of R^3 and R^4 comprises the *in vivo* imaging moiety.

In a most preferred embodiment, the *in vivo* imaging agent of the invention is a compound of Formula I*:

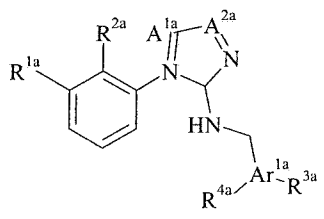


(I*)

wherein R^{1*} and R^{2*} are both halo, and R^{3*} is C_{1-3} alkyl, fluoro, iodo, or C_{1-3} fluoroalkoxy, and A^{1*} and A^{2*} are as defined previously for A^1 and A^2, respectively.

The *in vivo* imaging agents of the invention are ligands for the $P2X_7$ receptor, and preferably demonstrate at least 70% inhibition of the function of an agonist to form a non-selective pore in HEK.293 cells (see Michel *et al*, B. J. Pharmacol. 1998; 125: 1194-1201). In terms of binding affinity, a ligand for the $P2X_7$ receptor has a K_d or K_i of between 0.01 and 100nM, preferably between 0.01 and 10nM, and most preferably between 0.01 and 1nM (as measured by: Humphreys *et al* 1998 Molecular Pharmacology, 54:22–32; Chessell *et al* 1998 British Journal of Pharmacology, 124: 1314–1320). In conjunction with binding affinity for the $P2X_7$ receptor, the *in vivo* imaging agents of the invention preferably have no affinity up to 10μM for other P2 receptors. The *in vivo* imaging agent of the invention is preferably an antagonist for the $P2X_7$ receptor.

The *in vivo* imaging agent of the invention may be obtained by reaction of a suitable source of the desired *in vivo* imaging moiety with a non-radioactive precursor compound of Formula Ia:



(Ia)

wherein one of R^{1a} to R^{4a} comprises a precursor group and the remainder of R^{1a} to R^{4a} are as defined above for R^1 to R^4 of Formula I, respectively and optionally comprise a

protecting group;

A^{1a} and A^{2a} are as defined above for A^1 and A^2 of Formula I, respectively;

Ar^{1a} is as defined above for Ar^1 of Formula I.

A "suitable source" of said *in vivo* imaging moiety means a chemically reactive form of said *in vivo* imaging moiety. Reaction of the suitable source of said *in vivo* imaging moiety with the precursor compound preferably leads to the formation of the desired *in vivo* imaging agent of the invention, without requiring any further steps.

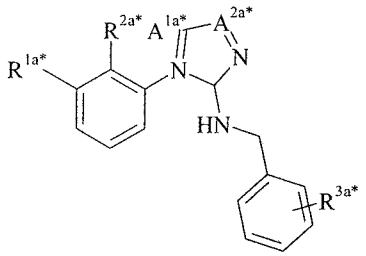
A "precursor compound" comprises an unlabelled, non-radioactive derivative of a compound of Formula I as defined above, i.e. the precursor compound comprises neither a gamma-emitting radioactive halogen nor a positron-emitting radioactive non-metal. The precursor compound is designed so that chemical reaction with a convenient chemical form of the imaging moiety occurs site-specifically; can be conducted in the minimum number of steps (ideally a single step); and without the need for significant purification (ideally no further purification), to give the desired *in vivo* imaging agent of Formula I as defined herein. Such precursor compounds are synthetic and can conveniently be obtained in good chemical purity. The precursor compound may optionally comprise a protecting group for certain functional groups of the precursor compound.

By the term "protecting group" is meant a group which inhibits or suppresses undesirable chemical reactions, but which is designed to be sufficiently reactive that it may be cleaved from the functional group in question under mild enough conditions that do not modify the rest of the molecule. After deprotection, the desired *in vivo* imaging agent of Formula I as defined herein is obtained. Protecting groups are well known to those skilled in the art and are suitably chosen from, for amine groups: BOC (where BOC is *tert*-butyloxycarbonyl), Fmoc (where Fmoc is fluorenylmethoxycarbonyl), trifluoroacetyl, allyloxycarbonyl, Dde [i.e. 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl] or Npys (i.e. 3-nitro-2-pyridine sulfenyl); and for carboxyl groups: methyl ester, *tert*-butyl ester or benzyl ester. For hydroxyl groups, suitable protecting groups are: methyl, ethyl or *tert*-butyl; alkoxymethyl or alkoxyethyl; benzyl;

acetyl; benzoyl; trityl (Trt) or trialkylsilyl such as tetrabutyldimethylsilyl. For thiol groups, suitable protecting groups are: trityl and 4-methoxybenzyl. The use of further protecting groups are described in 'Protective Groups in Organic Synthesis', Theorodora W. Greene and Peter G. M. Wuts, (Fourth Edition, John Wiley & Sons, 2007).

A "precursor group" is a chemical group which reacts with a convenient chemical form of the imaging moiety to incorporate the imaging moiety site-specifically. Suitable such precursor groups are discussed in more detail below. For example, such precursor groups include, but are not limited to, iodo, hydroxyl, nitro, iodonium salt, bromo, mesylate, tosylate, trialkyltin, $B(OH)_2$, and trialkylammonium salt.

In a preferred embodiment, the precursor compound of Formula Ia is a compound of Formula Ia^*:



(Ia^*)

wherein one of R^{1a*} to R^{3a*} comprises a precursor group and wherein the rest of R^{1a*} to R^{3a*} are as defined above for R^{1a} to R^{3a}, respectively, and A^{1a*} and A^{2a*} are as defined above for A^{1a} and A^{2a}, respectively.

Examples of precursor compounds suitable for incorporating representative *in vivo* imaging moieties of the present invention are now described.

Where the imaging moiety is radioiodine, the *in vivo* imaging agent as defined herein can be obtained by means of a precursor compound comprising a precursor group which either undergoes electrophilic or nucleophilic iodination or undergoes condensation with a labelled aldehyde or ketone. Examples of the first category are:

(a) organometallic derivatives such as a trialkylstannane (e.g. trimethylstannyl or

tributylstannyl), or a trialkylsilane (e.g. trimethylsilyl) or an organoboron compound (e.g. boronate esters or organotrifluoroborates);

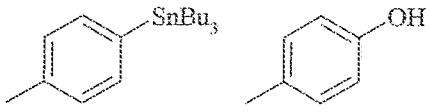
(b) a non-radioactive alkyl bromide for halogen exchange or alkyl tosylate, mesylate or triflate for nucleophilic iodination;

(c) aromatic rings activated towards nucleophilic iodination (e.g. aryl iodonium salt aryl diazonium, aryl trialkylammonium salts or nitroaryl derivatives).

Preferred such precursor compounds comprise precursor groups selected from: a non-radioactive halogen atom such as an aryl iodide or bromide (to permit radioiodine exchange); an organometallic precursor group (e.g. trialkyltin, trialkylsilyl or organoboron compound); or an organic precursor group such as triazenes, or a precursor group which is a good leaving group for nucleophilic substitution such as an iodonium salt.

Precursor compounds and methods of introducing radioiodine into organic molecules are described by Bolton (J. Lab. Comp. Radiopharm., 2002; 45: 485-528). Suitable boronate ester organoboron compounds and their preparation are described by Kabalka *et al* (Nucl. Med. Biol., 2002; 29: 841-843, and Nuc. Med Biol. 2003; 30: 369-373). Suitable organotrifluoroborates and their preparation are described by Kabalka *et al* (Nucl. Med. Biol. 2004; 31: 935-938).

Examples of aryl groups to which radioactive iodine can be attached are given below:



Both contain precursor groups which permit facile radioiodine substitution onto the aromatic ring.

Alternatively, *in vivo* imaging agents containing radioactive iodine can be synthesised by direct iodination *via* radiohalogen exchange, e.g.

The radioiodine atom is preferably attached *via* a direct covalent bond to an aromatic ring such as a benzene ring, or a vinyl group since it is known that iodine atoms bound to saturated aliphatic systems are prone to *in vivo* metabolism and hence loss of the radioiodine.

Preferably for obtaining *in vivo* imaging agents of the present invention where the imaging moiety is radioiodine, the precursor compound comprises a precursor group which is an organometallic precursor group, most preferably trialkyltin.

Radiobromination can be achieved by methods similar to those described above for radioiodination. Kabalka and Varma have reviewed various methods for the synthesis of radiohalogenated compounds, including radiobrominated compounds (Tetrahedron 1989; 45(21): 6601-21).

One approach to labelling with ^{11}C is to react a precursor compound which is the desmethylated version of a methylated compound with [^{11}C]methyl iodide. It is also possible to incorporate ^{11}C by reacting a Grignard reagent of the particular hydrocarbon of the desired *in vivo* imaging agent with [^{11}C]CO_2 to obtain a ^{11}C reagent that reacts with an amine group in the precursor compound to result in the ^{11}C-labelled *in vivo* imaging agent of interest.

^{11}C could also be introduced as a methyl group on an aromatic ring, in which case the precursor compound would include a precursor group that is a trialkyltin group or a $B(OH)_2$ group.

As the half-life of ^{11}C is only 20.4 minutes, it is important that the intermediate ^{11}C moieties have high specific activity and, consequently, that they are produced using a reaction process which is as rapid as possible.

A thorough review of such ^{11}C-labelling techniques may be found in Antoni *et al* "Aspects on the Synthesis of ^{11}C-Labelled Compounds" in Handbook of Radiopharmaceuticals, M.J. Welch and C.S. Redvanly Eds. (2003, John Wiley and

Sons).

Preferably for obtaining *in vivo* imaging agents of the present invention where the imaging moiety is ^{11}C, the precursor compound comprises a precursor group which is trialkyltin group or a $B(OH)_2$, most preferably trialkyltin.

Radiofluorination may be carried out *via* direct labelling using the reaction of ^{18}F-fluoride with a suitable chemical group in a precursor compound having a good leaving group, such as an alkyl bromide, alkyl mesylate or alkyl tosylate. For aryl systems, ^{18}F-fluoride nucleophilic displacement from an aryl diazonium salt, aryl nitro compound or an aryl quaternary ammonium salt are suitable routes to aryl-^{18}F derivatives.

Further details of synthetic routes to ^{18}F-labelled derivatives are described by Bolton (J. Lab. Comp. Radiopharm., 2002; 45: 485-528).

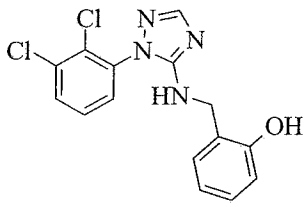
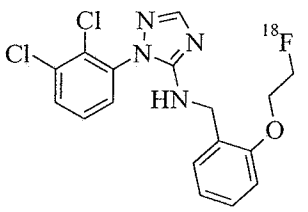
When the *in vivo* imaging moiety is a radioactive isotope of fluorine the radiofluorine atom may form part of a fluoroalkyl or fluoroalkoxy group, since alkyl fluorides are resistant to *in vivo* metabolism. Alternatively, the radiofluorine atom may attach *via* a direct covalent bond to an aromatic ring such as a benzene ring.

^{18}F can be introduced by O-alkylation of hydroxyl precursor groups with $^{18}F(CH_2)_3OMs$ or $^{18}F(CH_2)_3Br$. For aryl systems, ^{18}F-fluoride nucleophilic displacement from an aryl group of a precursor group which is a diazonium salt, a nitro or a quaternary ammonium salt is a suitable route to obtain an aryl-^{18}F derivative. Radiofluorination may also be carried out *via* direct labelling using the reaction of [^{18}F]-fluoride with a precursor group which is a good leaving group, such as bromide, mesylate, triflate, or tosylate. In this way, the precursor compound may be labeled in one step by reaction with a suitable source of [^{18}F]-fluoride ion ($^{18}F^-$), which is normally obtained as an aqueous solution from the nuclear reaction $^{18}O(p,n)^{18}F$ and is made reactive by the addition of a cationic counterion and the subsequent removal of water. For this method, the precursor compounds are normally selectively chemically protected so that radiofluorination takes place at a particular site. Suitable protecting groups are those already mentioned previously.

Preferably for obtaining *in vivo* imaging agents of the present invention where the imaging moiety is ^{18}F, the precursor compound comprises a precursor group which is a leaving group, most preferably mesylate, triflate, or tosylate.

The preferred and most preferred compounds as defined above in connection with the method of the invention themselves form an additional aspect of the invention.

A particularly preferred *in vivo* imaging agent of the invention and a precursor compound that was used to obtain it (synthesis described in Example 2) are as follows:

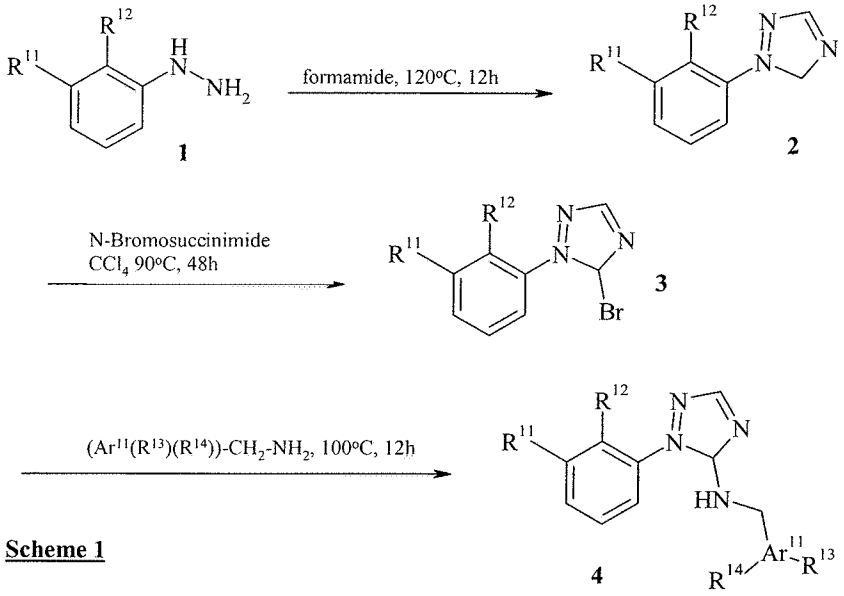
	
Precursor Compound 1	Imaging Agent 1

A non-radioactive analogue of the Imaging Agent illustrated in Table I was screened in a $P2X_7$ receptor functional assay. This assay is described in Example 3 and is based upon the ability of the $P2X_7$ receptor to form a non-selective pore in $P2X_7$ transfected HEK.293 cells upon activation with an agonist, thereby allowing dye to permeate the cells. The non selective P2X channel antagonist used as a reference inhibitor for the evaluation of the non-radioactive compound of the invention was pyridoxal-phosphate-6-azophenyl-2',4'-disulfonate (PPADS), and the results of the assay are provided in Table I above. The non-radioactive analogue of the imaging agent of the invention illustrated in Table I was found to inhibit $P2X_7$ function at 10 μM and generally at 100 nM concentrations to a similar degree compared to PPADS (the reference compound, which showed 70% inhibition at 10 μM).

The synthetic routes used to obtain Imaging Agent 1 illustrated in Table I, along with its non-radioactive analogue, are provided in Examples 1 and 2. Analogous methods can be used to obtain imaging agents over the whole scope of the claims. Precursors for the synthesis of *in vivo* imaging agents of the invention may be obtained using methods such as described by Florjancic *et al* (2008 Bioorg. Med. Chem. Lett., 18: 2089 and references cited therein). Starting compounds and intermediates are either

commercially available or described in Florjancic *et al* (supra) and/or the references cited therein.

To obtain precursor compounds suitable for preparing *in vivo* imaging agents of the invention where A^1 is N and A^2 is CH, the following generic reaction scheme may be used:

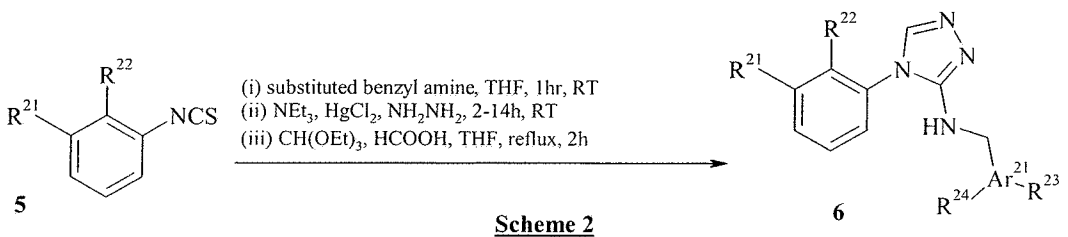


Scheme 1

In Scheme 1 above, R^{11} to R^{14} and Ar^{11} are as defined above for R^{1a} to R^{4a} and Ar^{1a}, respectively.

The appropriate phenylhydrazine **1** starting compound is reacted with formamide at elevated temperature to provide the triazole **2**, which is in turn brominated to provide intermediate **3**. Direct reaction of **3** with an appropriate benzyl amine results in **4**.

To obtain precursor compounds suitable for preparing *in vivo* imaging agents of the invention where A^1 is CH and A^2 is N, a slightly different generic reaction scheme is used as follows:



Scheme 2

In Scheme 2 above, R^{21} to R^{24} and Ar^{21} are as defined above for R^{1a} to R^{4a} and Ar^{1a}, respectively, NCS stands for *N*-Chlorosuccinimide, THF stands for tetrahydrofuran, RT stands for room temperature, and NEt_3 stands for triethylamine.

The starting material for Scheme 2 is the isothiocyanate compound **5**. Treatment of **5** with a benzyl amine in THF provides a thiourea intermediate, which, by addition of hydrazine in the presence of base and $HgCl_2$ gives the corresponding aminoguanidine. This is then heated to reflux in the presence of an orthoformate under acidic conditions to result in the product **6**.

The precursor compound for synthesising the imaging agent of the present invention may be conveniently provided as part of a kit, for example for use in a radiopharmacy. Such a kit comprises the precursor compound as defined herein in a sealed container. The sealed container preferably permits maintenance of sterile integrity and/or radioactive safety, plus optionally an inert headspace gas (e.g. nitrogen or argon), whilst permitting addition and withdrawal of solutions by syringe. A preferred sealed container is a septum-sealed vial, wherein the gas-tight closure is crimped on with an overseal (typically of aluminium). Such sealed containers have the additional advantage that the closure can withstand vacuum if desired e.g. to change the headspace gas or degas solutions.

Suitable and preferred embodiments of the precursor compound when employed in the kit of the invention are as already described herein.

The precursor compound for use in the kit may be employed under aseptic manufacture conditions to give the desired sterile, non-pyrogenic material. The precursor compound may alternatively be employed under non-sterile conditions, followed by terminal sterilisation using e.g. gamma-irradiation, autoclaving, dry heat or chemical treatment (e.g. with ethylene oxide). Preferably, the precursor compound is provided in sterile, non-pyrogenic form. Most preferably the sterile, non-pyrogenic precursor compound is provided in the sealed container as described above.

Preferably, all components of the kit are disposable to minimise the possibilities of contamination between runs and to ensure sterility and quality assurance.

In a preferred aspect, the method of synthesis of the present invention is automated. [^{18}F]-radiotracers in particular are now often conveniently prepared on an automated radiosynthesis apparatus. There are several commercially-available examples of such apparatus, including Tracerlab™ and Fastlab™ (both available from GE Heathcare). The radiochemistry is performed on the automated synthesis apparatus by fitting the cassette to the apparatus. The cassette normally includes fluid pathways, a reaction vessel, and ports for receiving reagent vials as well as any solid-phase extraction cartridges used in post-radiosynthetic clean up steps.

In a yet further aspect, the present invention provides a cassette which can be plugged into a suitably adapted automated synthesiser for the automated synthesis of the *in vivo* imaging agent of the invention.

The cassette for the automated synthesis of the *in vivo* imaging agent of the invention comprises:

(i) a vessel containing a precursor compound as defined herein; and

(ii) means for eluting the vessel with a suitable source of an *in vivo* imaging moiety, said *in vivo* imaging moiety as defined herein.

The cassette may additionally comprise:

(iii)an ion-exchange cartridge for removal of excess *in vivo* imaging moiety; and optionally,

(iv)a cartridge for deprotection of the resultant radiolabelled product to form an *in vivo* imaging agent as defined herein.

The reagents, solvents and other consumables required for the synthesis may also be included together with a data medium, such as a compact disc carrying software, which allows the automated synthesiser to be operated in a way to meet the end user's requirements for concentration, volumes, time of delivery etc.

The *in vivo* imaging agent of the invention is particularly useful for the assessment by *in vivo* imaging of the number and/or location of $P2X_7$ receptors in the CNS of a subject.

In a further aspect therefore, the present invention provides a method of imaging a subject to facilitate the determination of the presence, location and/or amount of $P2X_7$ receptors in the CNS of a subject, said method comprising the following steps:

(i) providing a subject to whom a detectable quantity of the *in vivo* imaging agent of the invention has been administered;

(ii) allowing the *in vivo* imaging agent to bind to $P2X_7$ receptors in said subject;

(iii) detection of signals emitted by said *in vivo* imaging agent by an *in vivo* imaging method; and,

(iv) generation of an image representative of the location and/or amount of said signals.

The method of the invention begins by "providing" a subject to whom a detectable quantity of an *in vivo* imaging agent of the invention has been administered. Since the ultimate purpose of the method is the provision of a diagnostically-useful image, administration to the subject of the *in vivo* imaging agent of the invention can be understood to be a preliminary step necessary to facilitate generation of said image.

In an alternative embodiment, step (i) of the method of imaging of the invention can instead be:

(i) administration to said subject of a detectable quantity of the *in vivo* imaging agent of the invention.

"Administration" of the *in vivo* imaging agent is preferably carried out parenterally, and most preferably intravenously. The intravenous route represents the most efficient way to deliver the *in vivo* imaging agent throughout the body of the subject, and therefore across the blood-brain barrier (BBB) and into the central nervous system (CNS) of said subject. Intravenous administration does not represent a substantial physical intervention or a substantial health risk. The *in vivo* imaging agent of the invention is preferably administered as the pharmaceutical composition of the invention, as defined herein.

A "detectable quantity" of an *in vivo* imaging agent is an amount that comprises

sufficient detectable label to enable signals emitted by the *in vivo* imaging moiety, following administration of said *in vivo* imaging agent to said subject, to be detected by the imaging apparatus.

The properties of the *in vivo* imaging agent of the invention make it suitable for crossing the BBB and binding to $P2X_7$ receptors within the CNS. Therefore, in the method of the invention the detection and generation steps are carried out on the CNS of said subject, preferably the brain.

The method of the invention may be used to study the location and/or amount of $P2X_7$ receptor in a healthy subject. However, the method is particularly useful when said subject is known or suspected to have a pathological condition associated with abnormal expression of $P2X_7$ receptors in the CNS (a "$P2X_7$ condition"). Such conditions include stroke, multiple sclerosis, amyotrophic lateral sclerosis, epilepsy, and Alzheimer's disease, and the pathophysiology of each comprises neuroinflammation. The term "neuroinflammation" refers to the fundamentally inflammation-like character of microglial and astrocytic responses and actions in the CNS. These responses are central to the pathogenesis and progression of a wide variety of neurological disorders including stroke, epilepsy, Parkinson's disease, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), Alzheimer's disease and Huntington's disease. Consequently, the image generated by the method of the invention finds use in providing guidance to a clinician in the diagnosis of such disorders.

In an alternative aspect, the present invention provides a method of diagnosis, comprising steps (i)-(iv) of the *in vivo* imaging method as defined above, and further comprising the following step:

(ii) evaluating the image generated in step (iv) to diagnose a pathological condition associated with abnormal expression of $P2X_7$ receptors in the CNS (a "$P2X_7$ condition").

The $P2X_7$ condition of step (v) is any one of those described herein. The evaluating step is carried out by a doctor or a vet, i.e. a person suitably qualified to make a clinical diagnosis. Such a diagnosis represents a deductive medical or veterinary decision, which is made for

the purpose of making a decision about whether any treatment is required to restore the subject to health.

In a further alternative embodiment, the method may include the preliminary step of administering the *in vivo* imaging agent of the invention to the subject. Administration of the *in vivo* imaging agent of the invention is preferably carried out parenterally, and most preferably intravenously. The intravenous route represents the fastest way of delivering the *in vivo* imaging agent of the invention across the BBB and into contact with $P2X_7$ receptors in the CNS. Preferred embodiments of said *in vivo* imaging agent and subject are as previously defined.

The *in vivo* imaging agent of the invention is preferably administered as a "radiopharmaceutical composition" which comprises the *in vivo* imaging agent of Formula I together with a biocompatible carrier, in a form suitable for mammalian administration.

The "biocompatible carrier" is a fluid, especially a liquid, in which the *in vivo* imaging agent of Formula I is suspended or dissolved, such that the radiopharmaceutical composition is physiologically tolerable, i.e. can be administered to the mammalian body without toxicity or undue discomfort. The biocompatible carrier medium is suitably an injectable carrier liquid such as sterile, pyrogen-free water for injection; an aqueous solution such as saline (which may advantageously be balanced so that the final product for injection is either isotonic or not hypotonic); an aqueous solution of one or more tonicity-adjusting substances (e.g. salts of plasma cations with biocompatible counterions), sugars (e.g. glucose or sucrose), sugar alcohols (e.g. sorbitol or mannitol), glycols (e.g. glycerol), or other non-ionic polyol materials (e.g. polyethyleneglycols, propylene glycols and the like). The biocompatible carrier medium may also comprise biocompatible organic solvents such as ethanol. Such organic solvents are useful to solubilise more lipophilic compounds or formulations. Preferably the biocompatible carrier medium is pyrogen-free water for injection, isotonic saline or an aqueous ethanol solution. The pH of the biocompatible carrier medium for intravenous injection is suitably in the range 4.0 to 10.5.

Such radiopharmaceutical compositions are suitably supplied in either a container which is

provided with a seal which is suitable for single or multiple puncturing with a hypodermic needle (e.g. a crimped-on septum seal closure) whilst maintaining sterile integrity. Such containers may contain single or multiple patient doses. Preferred multiple dose containers comprise a single bulk vial (e.g. of 10 to 30 cm^3 volume) which contains multiple patient doses, whereby single patient doses can thus be withdrawn into clinical grade syringes at various time intervals during the viable lifetime of the preparation to suit the clinical situation. Pre-filled syringes are designed to contain a single human dose, or "unit dose", and are therefore preferably a disposable or other syringe suitable for clinical use. The pre-filled syringe may optionally be provided with a syringe shield to protect the operator from radioactive dose. Suitable such radiopharmaceutical syringe shields are known in the art and preferably comprise either lead or tungsten.

The radiopharmaceutical composition may be prepared from a kit. Alternatively, they may be prepared under aseptic manufacture conditions to give the desired sterile product. The radiopharmaceutical composition may also be prepared under non-sterile conditions, followed by terminal sterilisation using e.g. gamma-irradiation, autoclaving, dry heat or chemical treatment (e.g. with ethylene oxide).

The method of imaging of the present invention may also be employed as a research tool. For example, for the performance of competition studies which allow the interaction of a drug with $P2X_7$ receptors to be studied. Such studies include dose-occupancy studies, determination of optimal therapeutic dose, drug candidate selection studies, and determination of $P2X_7$ receptor distribution in the tissue of interest.

In an alternative embodiment, the method of the invention is effected repeatedly, e.g. before, during and after treatment with a drug to combat a $P2X_7$ condition. In this way, the effect of said treatment can be monitored over time.

Also provided by the present invention is an *in vivo* imaging agent of the invention for use in medicine, and in particular for use in a method for the determination of the presence, location and/or amount of inflammation in the CNS of a subject. Suitable and preferred embodiments of said *in vivo* imaging agent, method and subject are as previously defined.

In a further aspect of the invention, the *in vivo* imaging agent of the invention may be

employed for use in the preparation of a medicament for the determination of the presence, location and/or amount of inflammation in the CNS of a subject. Suitable and preferred embodiments of said *in vivo* imaging agent and said subject are as previously defined herein.

Detailed methods for the synthesis of particular *in vivo* imaging agents of the invention are provided in the following non-limiting Examples.

Brief Description of the Examples

Example 1 describes the synthesis of a non-radioactive analogue of imaging agent 1.

Example 2 describes the synthesis of imaging agent 1.

Example 3 describes the assay used to evaluate binding to the $P2X_7$ receptor.

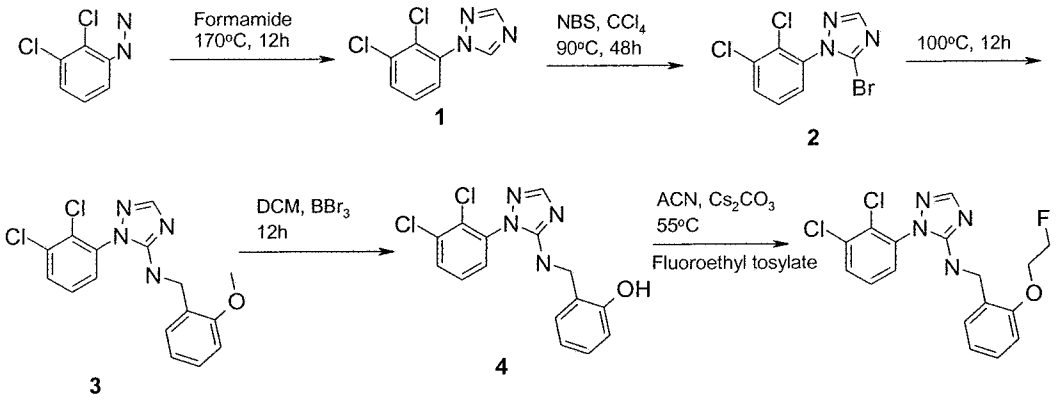
Abbreviations used in the Examples

AIBN	azobisisobutyronitrile
ATP	adenosine triphosphate
BOC	*tert*-butoxycarbonyl
Bz-ATP	2' and 3'-O-(4-benzoylbenzoyl)-ATP
DEAD	diethyl azodicarboxylate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC	high-performance liquid chromatography
IC50	half maximal inhibitory concentration

LDA	lithium diisopropylamide
MeOH	methanol
NBS	*N*-bromosuccinimide
PPADs	pyrdoxalphosphate-6-azophenyl-2'4'-disulphonic acid
RNA	ribonucleic acid
RT	room temperature
THF	tetrahydrofuran

Examples

Example 1: Synthesis of a Non-radioactive Analogue of Imaging Agent 1 (1-(2,3-dichlorophenyl)-N- (2-(2-fluoroethoxy) benzyl)-1H-1, 2,4-triazol-5-amine)



1(i) 1-(2,3-Dichlorophenyl)-1H-1, 2, 4-triazole (1)

Added 2,3-dichlorophenyhydrazine (2.5 g, 14.12 mmol) to an oven dried flask. To this was added formamide (15 mL). The mixture was kept at 170°C for 12h. The reaction mass was quenched with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic layers were then washed with water (4 x 25 mL) and dried over anhydrous sodium sulphate, filtered and evaporated. The crude reaction mass was purified through column chromatography on silica gel using hexane and ethyl acetate as

an eluent to give the product (1.8 g, 60% yield) as a white chalky solid.

^{1}H-NMR: (400 MHz, $CDCl_3$) δ 8.55 (s, 1H), 8.17 (s, 1H), 7.64 (d, 1H, J=8Hz), 7.52 (d, 1H, J=8Hz), 7.40 (t, 1H, J=8Hz).

1(ii) 5-Bromo-1-(2,3-dichlorophenyl)-1H-[1,2,4]-triazole (2)

1-(2,3-Dichlorophenyl)-1H-[1,2,4]-triazole (**1**), (1.0 g 4.67 mmol) was taken in a round bottom oven dried flask. To this was added anhydrous carbon tetrachloride (15 mL) followed by freshly crystallized NBS (1.1 g, 6.18 mmol) and a catalytic amount of AIBN. The reaction was then heated to reflux at 90°C for about 48h. The carbon tetrachloride was removed under reduced pressure and the reaction mass was purified by column chromatography on silica gel using hexane and ethyl acetate as the solvent to give the product **2** (900 mg, 65% yield).

^{1}H-NMR: (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.72 (d, 1H, J=8Hz), 7.41 (q, 2H, J=8Hz).

1(iii) 1-(2,3-Dichlorophenyl)-N- (2-methoxybenzyl)-1H-[1,2,4]-triazol-5-amine (3)

Both the starting material **2** (500 mg, 1.7 mmol) and 2-methoxybenzylamine (0.5 mL) were taken in a round bottom flask. and heated to reflux at 100°C for 12h. The reaction was then quenched with water (20 mL) and extracted with ethyl acetate (3 x 20 mL). The combined extracts were washed with brine (15 mL), dried over anhydrous sodium sulphate, filtered and evaporated. The product was isolated by column chromatography on silica gel using hexane and ethyl acetate as eluent to give **3** (500 mg, 84% yield).

^{1}H-NMR: (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.62 (t, 1H, J=4Hz), 7.25-7.41 (m, 4H), 6.94 (t, 1H, J=8Hz), 6.88 (d, 1H, J=8Hz), 4.68 (s, 1H), 4.60 (s, 2H), 3.8 (s, 3H).

1(iv) 2-((1-(2,3-Dichlorophenyl)-1H-[1,2,4]-triazol-5-yl-amino) methyl) phenol (4)

1-(2,3-Dichlorophenyl)-N- (2-methoxybenzyl)-1H-[1,2,4]-triazol-5-amine (**3**) (500 mg, 1.4 mmol) was dissolved in anhydrous dichloromethane (5 mL) in a dried flask. The reaction mass was then cooled to -78°C and stirred for 15 minutes. Boron tribromide (0.4ml, 1.6 mmol) was then added while maintaining the same temperature. The reaction was allowed to come to room temperature and stirred for a total of about 12h

before being quenched with water (20 mL slow addition) and extracted with dichloromethane (3 x 20 mL) and purified by column chromatography on silica gel using hexane – ethyl acetate eluent to give the product (160 mg, 33%yield).

^{1}H-NMR: (400 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.65 (m, 1H), 7.38 (d, 2H, J=4Hz), 7.25 (t, 1H, J=8Hz), 7.14 (d, 1H, J=8Hz), 7.01 (d, 1H, J=8Hz), 6.88 (t, 1H, J=8Hz), 4.76 (s, 1H), 4.50 (s, 2H).

1(v) (1-(2,3-dichlorophenyl)-N- (2-(2-fluoroethoxy) benzyl)-1H-[1,2,4]-triazol-5-amine) (Non-radioactive Analogue of Imaging Agent 1)

4 (160 mg, 0.48 mmol) was dissolved in acetonitrile (2 mL) and cesium carbonate (1.2eqv) was added and mixture then stirred for 15 minutes at room temperature. Fluoroethyl tosylate (1.1eqv) was added to this mixture and the reaction heated at 55°C for 12h. Acetonitrile was then removed under reduced pressure and the residue partitioned between ethyl acetate and water. The organic layer was then concentrated and purified by column chromatography on silica gel using hexane and ethyl acetate as eluent to give the desired product (96 mg, 53% yield).

^{1}H-NMR: (400 MHz, $CDCl_3$) δ 7.50-7.82 (m, 2H), 7.11-7.49 (m, 5H), 6.98 (t, 1H, J=8Hz), 6.84 (d, 1H, J=8Hz), 4.62 (m, 5H), 4.17 (m, 2H).

Example 2: Synthesis of Imaging Agent 1

Imaging Agent 1 is obtained using the method as described in Example 1 except that **4** is reacted with [^{18}F]-Fluoroethyl tosylate (synthesised e.g. as described by Bauman *et al* Tetrahedron Letts. 2003; 44: 9165-7) in acetonitrile in the presence of potassium carbonate and Kryptofix.

Example 3: Pore-forming Assay to determine $P2X_7$ Binding

The assay method used was based on the ability of the DNA binding dye, Yo Pro-1 (quinolinium, 4[3-methyl-2(3H)-benzoxazolylidene) methyl]-1-[3-(trimethyl-ammonio) propyl]-dioxide) to enter through the dilated or "large pore form" of the $P2X_7$ receptor

and to bind to intracellular DNA/RNA whereupon it increases fluorescence intensity. Yo Pro-1 was therefore used to quantify inhibition of $P2X_7$ function.This assay was based on the methods published by Michel *et al.*, (B.J.Pharmacol 1998; 125: 1194-1201).

Initially, HEK.293 cells were transiently transfected using LipofectamineTMLTX (Invitrogen) for 72hrs with $P2X_7$ cDNA. 48 hours prior to use the cells were seeded into poly-D-lysine coated 96-well black-walled, clear bottomed plates, at a density of 30,000 cells/well. Stock solutions of test compound were prepared at a concentration of 40mM in 100% DMSO.

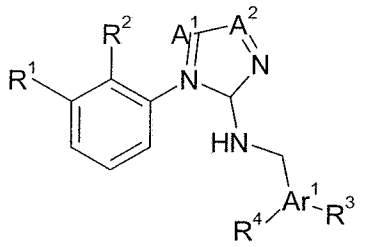
Following the 48 hour incubation the culture medium was removed from the transfected cells, the cells were washed once and placed in pre-warmed sucrose assay buffer (Sucrose: 280mM, KCl: 5mM, CaCl2: 0.5mM, glucose: 10mM, HEPES: 10mM, N-methyl-D-glucamine: 10mM; pH7.4). The test compounds were added to the plate at a concentration of 10μM and 100nM in triplicate and incubated at 37°C for 30 minutes. The final DMSO concentration in the assay was 1%. After this time Yo Pro-1 dye and Bz-ATP solution was added at concentrations of 1μM and 30μM respectively for 60 minutes at 37°C. The fluorescence was then read at 485 nM excitation and 530 nM emission.

The non-selective P2X channel antagonist pyrdoxalphosphate-6-azophenyl-2'4'-disulphonic acid (PPADS) was used as a reference inhibitor in the assay. A dose-response to PPADS was performed on the assay plate using a starting concentration of 200μM followed by a 1 in 6 serial dilution covering the concentration range 200μM to 0.4nM. For each compound data set, a percentage inhibition value was calculated based on the three assay points generated. For imaging agent 1 % inhibition was found to be 77.0 at 10μM and 68.0 at 100μM

Claims

1) An *in vivo* imaging agent comprises a compound of Formula I, or a salt or solvate thereof, wherein:

Formula I is defined as follows:



(I)

wherein:

R^1 and R^2 are independently selected from hydrogen, halo, hydroxyl, C_{1-3} alkyl, C_{1-3} fluoroalkyl, and C_{1-3} hydroxyalkyl;

R^3 and R^4 are independently selected from hydrogen, halo, hydroxyl, C_{1-3} alkyl, C_{1-3} fluoroalkyl, C_{1-3} hydroxyalkyl, C_{1-3} alkyloxy, C_{1-3} fluoroalkyloxy, C_{1-3} alkylthio, C_{1-3} fluoroalkylthio and C_{1-6} cycloalkyl;

one of A^1 and A^2 is N and the other is CH;

Ar^1 is a C_{5-12} aryl group optionally comprising 1-3 heteroatoms selected from nitrogen, oxygen and sulfur; and,

wherein any one of R^1, R^2, R^3 and R^4 as defined comprises an *in vivo* imaging moiety which is a gamma-emitting radioactive halogen or a positron-emitting radioactive non-metal.

2) The *in vivo* imaging agent as defined in Claim 1 wherein R^1 and R^2 are independently selected from hydrogen, halo, and hydroxyl.

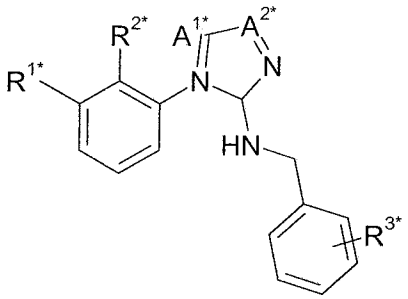
3) The *in vivo* imaging agent as defined in either Claim 1 or Claim 2 wherein R^3 and R^4 are independently selected from hydrogen, hydroxyl, halo, and C_{1-3} fluoroalkoxy.

4) The *in vivo* imaging agent as defined in any one of Claims 1-3 wherein A^1 is N and A^2 is CH.

5) The *in vivo* imaging agent as defined in any one of Claims 1-4 wherein Ar^1 is a C_{5-6} aryl group optionally comprising 1 heteroatom selected from nitrogen, oxygen and sulfur.

6) The *in vivo* imaging agent as defined in any one of Claims 1-5 wherein one of R^3 and R^4 comprises said *in vivo* imaging moiety.

7) The *in vivo* imaging agent as defined in any one of Claims 1-6, which is a compound of Formula I^*:



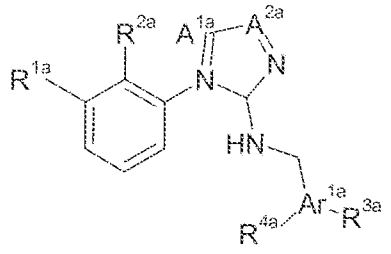
(I^*)

wherein R^{1*} and R^{2*} are both halo, and R^{3*} is C_{1-3} alkyl, fluoro, iodo, or C_{1-3} fluoroalkoxy, and A^{1*} and A^{2*} are as defined in Claim 1 or Claim 4 for A^1 and A^2, respectively.

8) The *in vivo* imaging agent as defined in any one of Claims 1-7 wherein said *in vivo* imaging moiety is selected from ^{123}I, ^{11}C and ^{18}F.

9) The *in vivo* imaging moiety as defined in any one of Claims 1-8 wherein said *in vivo* imaging moiety is ^{18}F.

10) A method for the synthesis of an *in vivo* imaging agent as defined in any one of Claims 1-9, wherein said method comprises reaction of a suitable source of said *in vivo* imaging moiety with a non-radioactive precursor compound of Formula Ia:



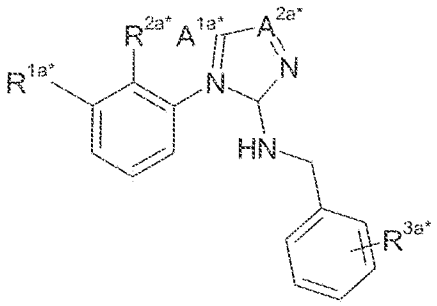
(Ia)

wherein one of R^{1a} to R^{4a} comprises a precursor group and the remainder of R^{1a} to R^{4a} are as defined in any one of Claims 1-3 for R^1 to R^4, respectively, and optionally comprise a protecting group;

A^{1a} and A^{2a} are as defined in Claim 1 or Claim 4 for A^1 and A^2, respectively; and,

Ar^{1a} is as defined in Claim 1 or Claim 5 for Ar^1.

11) The method as defined in Claim 10 wherein said precursor compound of Formula Ia is a compound of Formula Ia*:



(Ia*)

wherein one of R^{1a*} to R^{3a*} comprises a precursor group and wherein the rest of R^{1a*} to R^{3a*} are as defined in Claims 10 for R^{1a} to R^{3a}, respectively, and A^{1a*} and A^{2a*} are as defined in Claim 10 or Claim 4 for A^{1a} and A^{2a}, respectively.

12) The method as defined in Claim 10 or Claim 11 wherein said method is automated.

13) The method as defined in any one of Claims 10-12 wherein said precursor group is selected from a trialkyltin group, $B(OH)_2$, mesylate, triflate, or tosylate.

14) A precursor compound as defined in the method of Claim 13.

15) A cassette for carrying out the method as defined in Claim 12 comprising:

(i) a vessel containing a precursor compound as defined in the method of any one of Claims 10-13; and

(ii) means for eluting the vessel with a suitable source of an *in vivo* imaging moiety, said *in vivo* imaging moiety as defined in any one of Claims 1, 8 or 9.

16) The cassette as defined in Claim 15 which additionally comprises:

(iii) an ion-exchange cartridge for removal of excess *in vivo* imaging moiety; and optionally,

(iv) a cartridge for deprotection of the resultant radiolabelled product to form an *in vivo* imaging agent as defined in any one of Claims 1-9.

17) A radiopharmaceutical composition which comprises the *in vivo* imaging agent as defined in any one of Claims 1-9, together with a biocompatible carrier, in a form suitable for mammalian administration.

18) A method of *in vivo* imaging a subject to facilitate the determination of the presence, location and/or amount of $P2X_7$ receptors in the CNS of a subject, said method comprising the following steps:

(i) providing a subject to whom a detectable quantity of an *in vivo* imaging agent as defined in any one of Claims 1-9 has been administered;

(ii) allowing the administered *in vivo* imaging agent to bind to $P2X_7$ receptors in said subject;

(iii) detection of signals emitted by said *in vivo* imaging agent by an *in vivo* imaging method; and,

(iv) generation of an image representative of the location and/or amount of said signals.

19) The method as defined in Claim 18 wherein said subject is an intact mammalian body *in vivo*.

20) The method as defined in Claim 18 or Claim 19 wherein said subject is known or suspected to have a pathological condition associated with abnormal expression of $P2X_7$ receptors in the CNS.

21) A method of diagnosis comprising steps (i)-(iv) as defined in Claim 18, and further comprising the following step:

(v) evaluating the image generated in step (iv) to diagnose a pathological condition associated with abnormal expression of $P2X_7$ receptors in the CNS (a "$P2X_7$ condition").

22) The *in vivo* imaging agent as defined in any one of Claims 1-9 for use in the method of diagnosis as defined in Claim 21.

23) The *in vivo* imaging agent as defined in any one of Claims 1-9 for use in the preparation of a medicament for a method of diagnosis as defined in Claim 21.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/054517

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K51/04 C07D249/06
ADD. A61K101/00 A61K101/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2007/141267 A1 (GLAXO GROUP LTD [GB]; BESWICK PAUL JOHN [GB]; CHAMBERS LAURA J [GB]; D) 13 December 2007 (2007-12-13) cited in the application page 2, lines 1-22 page 7, lines 1-27	1-23
Y	WO 2008/064432 A1 (UNIV SYDNEY [AU]; KASSIOU MICHAEL [AU]; COSTER MARK [AU]; GUNOSEWOYO H) 5 June 2008 (2008-06-05) cited in the application page 27, lines 19-33 claim 1	1-23
Y	WO 2007/056046 A1 (ABBOTT LAB [US]; CARROLL WILLIAM A [US]; FLORJANCIC ALAN S [US]; PEREZ) 18 May 2007 (2007-05-18) examples	1-23

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 July 2010	06/08/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Villard, Anne-Laure

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FLORJANCIC A S ET AL: "Synthesis and in vitro activity of 1-(2,3-dichlorophenyl)-N-(pyridin-3-ylmethyl)-1H-1,2,4-triazol-5-amine and 4-(2,3-dichlorophenyl)-N-(py ridin-3-ylmethyl)-4H-1,2,4-triazol-3-amine P2X7 antagonists" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, ELSEVIER SCIENCE, GB, vol. 18, no. 6, 15 March 2008 (2008-03-15) , pages 2089-2092, XP025695025 ISSN: 0960-894X [retrieved on 2008-01-30] cited in the application the whole document --- WO 2009/106564 A2 (GE HEALTHCARE LTD [GB]; JONES PAUL ALEXANDER [GB]; WILSON IAN [GB]; MO) 3 September 2009 (2009-09-03) page 18, imaging agents 4 and 5 claims ---	1-23
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2010/054517

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