(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 October 2012 (04.10.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/131037 A1

(51) **International Patent Classification**:
C07B 59/00 (2006.01) ***A61K 101/02*** (2006.01)
A61K 51/04 (2006.01) ***C07D 487/14*** (2006.01)

(21) **International Application Number**:
PCT/EP2012/055789

(22) **International Filing Date**:
30 March 2012 (30.03.2012)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
1105445.9 31 March 2011 (31.03.2011) GB
61/469,847 31 March 2011 (31.03.2011) US

(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)*: **SOLBAKKEN, Magne** [NO/NO]; GE Healthcare AS, Nycoveien 2, Postboks 4220 Nydalen, N-0401 Oslo (NO). **GAETA, Alessandra** [IT/GB]; GE Healthcare Limited, The Grove Centre, White Lion Road, Amersham Buckinghamshire HP7 9LL (GB). **O'SHEA, Dennis** [AU/GB]; GE Healthcare Limited, The Grove Centre, White Lion Road, Amersham Buckinghamshire HP7 9LL (GB). **JACKSON, Alexander** [GB/GB]; GE Healthcare Limited, The Grove Centre, White Lion Road, Amersham Buckinghamshire HP7 9LL (GB). **TRIGG, William, John** [GB/GB]; GE Healthcare Limited, The Grove Centre, White Lion Road, Amersham Buckinghamshire HP7 9LL (GB).

(74) **Agents**: **BANNAN, Sally** et al.; GE Healthcare Limited, Pollards Wood, Nightingales Lane, Chalfont St Giles Buckinghamshire HP8 4SP (GB).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2012/131037 A1

(54) **Title**: RADIOLABELLED FLUMAZENIL DERIVATIVES

(57) **Abstract**: A novel 18F- labelled compound suitable for use as a PET tracer for imaging GABA receptors is provided as well as a precursor compound for use in its synthesis and methods for its preparation. Also disclosed herein is an in vivo imaging method comprising administration of said 18F- labelled compound, which finds use in the diagnosis of conditions in which expression of the GABAA receptor is abnormal, or in the monitoring of such conditions.

Radiolabelled Flumazenil Derivatives

Technical Field of the Invention

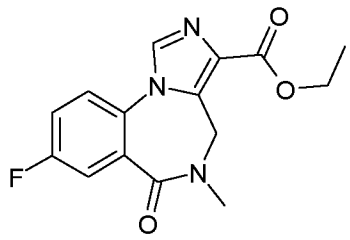
The present invention relates to *in vivo* imaging and in particular to *in vivo* imaging of gamma-aminobutyric acid (GABA) receptors of the central nervous system (CNS). The invention provides novel *in vivo* imaging agents that target GABA receptors.

Description of Related Art

Gamma-aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the human brain. GABA receptors are transmembrane receptors and fall into two main types, $GABA_A$ receptors and $GABA_B$ receptors. $GABA_A$ receptors have been the major focus of pharmacological development to date. Many $GABA_A$ receptor subtypes have been discovered and novel chemical structures have been developed which are selective for these subtypes. Normal activation of the $GABA_A$ receptor results in chloride ion being selectively conducted through its pore. This chloride channel gating is generally inhibitory on a neuron by virtue of stabilising the membrane potential near to resting level.

Defective $GABA_A$ receptor neurotransmission may be caused by a reduction in $GABA_A$ receptors, or by defective functioning of the $GABA_A$ receptor due to e.g. a genetic mutation in a $GABA_A$ receptor gene, traumatic brain injury, or a pharmacological insult, and is implicated in a number of neurological and psychiatric disorders, including epilepsy, anxiety disorders, Parkinson's disease and chronic pain. The development of radioligands selective for the $GABA_A$ receptor is therefore of value in terms of brain imaging studies in living human patients, in particular those suffering from disorders associated with defective $GABA_A$ receptor neurotransmission.

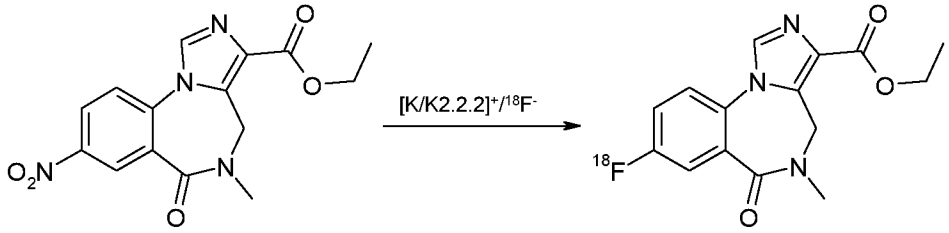
Flumazenil (also known as flumazepil, code name Ro 15-1788, trade names Anexate, Lanexat, Mazicon, Romazicon) is an imidazo[1,5-*a*][1,4]benzodiazepine that is a neutralising allosteric modulator of $GABA_A$ receptors in the CNS (Johnston 1996 Pharmacol Ther; 69(3): 173-198).



Flumazenil

The most common use of flumazenil to date has been as an antidote to benzodiazepine overdose as it reverses the effects of benzodiazepines by competitive inhibition at the benzodiazepine binding site of the $GABA_A$ receptor. In addition, because flumazenil has little or no agonist activity, radiolabelled versions thereof have been developed as positron emission tomography (PET) radiotracers.

[^{18}F]FMZ has the same chemical formula as flumazenil but wherein ^{18}F is incorporated by direct radiofluorination of a nitro precursor:



[^{18}F]Flumazenil

[^{18}F]FMZ binds to the $GABA_A$ receptor with high affinity (K_i around 0.5nM).

However, the above-illustrated radiolabelling process to obtain [^{18}F]FMZ from the corresponding 8-nitro precursor is low yielding for poor fluoride incorporation. Furthermore, there is scope to provide improved $GABA_A$-binding imaging agents that are cleared less quickly from the brain than [^{18}F]FMZ, and that have more activity left in the brain 30 minutes following administration. The present invention aims to provide an *in vivo* imaging agent that overcomes the problems associated with [^{18}F]FMZ.

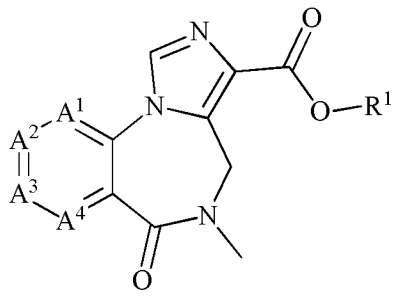
Summary of the Invention

The present invention provides a novel ^{18}F-labelled compound suitable for use as a PET tracer for imaging GABA receptors. Also provided by the present invention is a precursor compound for use in obtaining the ^{18}F-labelled compound of the invention and a method to prepare the ^{18}F-labelled compound of the invention comprising

radiofluorination of said precursor compound. Given that the ^{18}F-labelled compound of the invention is useful as a PET tracer for imaging GABA receptors, the present invention also provides an *in vivo* imaging method comprising administration of said ^{18}F-labelled compound. The *in vivo* imaging method of the invention may be usefully applied for the diagnosis of conditions in which expression of the $GABA_A$ receptor is abnormal, or in the monitoring of such conditions, particularly during the course of a treatment.

Detailed Description of the Invention

In one aspect, the present invention provides an ^{18}F-labelled compound of Formula I:



I

wherein one of A^1-A^4 is a nitrogen heteroatom, one of A^1-A^4 is C-^{18}F and the remaining two of A^1-A^4 are CH; and,

R^1 is a straight or branched C_{1-4} alkyl.

The term "^{18}F-labelled" refers to the fact that one atom of the compound of the invention is the radioactive isotope of fluorine, ^{18}F.

The term "nitrogen heteroatom" refers to a nitrogen atom that takes the place of a carbon atom in a hydrocarbon chain. In the context of Formula I the nitrogen heteroatom is present in the 6-membered aryl ring.

When one of A^1-A^4 is "C-^{18}F" this is taken to mean that one of A^1-A^4 is carbon with an ^{18}F attached directly thereto.

The term "straight or branched C_{1-4} alkyl" refers to any straight or branched C_nH_{2n+1} group wherein n is an integer from 1-4.

In one preferred embodiment of the ^{18}F-labelled compound of the invention, A^1 is a nitrogen heteroatom. Preferably for this embodiment, A^4 is C-^{18}F.

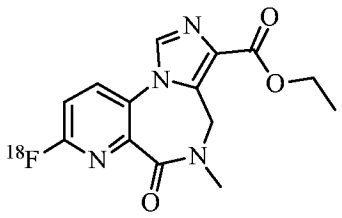
In another preferred embodiment of the ^{18}F-labelled compound of the invention, A^3 is a

nitrogen heteroatom. Preferably for this embodiment, A^4 is C-^{18}F.

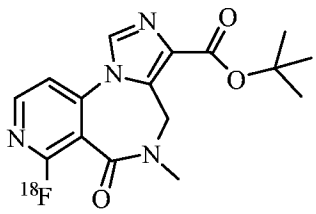
In yet another preferred embodiment of the ^{18}F-labelled compound of the invention, A^4 is a nitrogen heteroatom. Preferably for this embodiment, A^3 is C-^{18}F.

Preferably for any of the above-described embodiments of the ^{18}F-labelled compound of the invention, R^1 is a straight or branched C_{2-4} alkyl. Most preferably, R^1 is selected from ethyl, isopropyl and *t*-butyl. Most especially preferably, R^1 is ethyl or *t*-butyl.

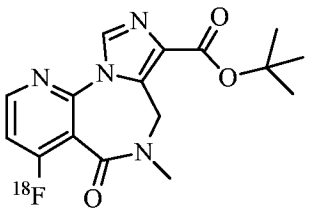
Non-limiting examples of preferred ^{18}F-labelled compounds of the present invention are the following:



Compound 1

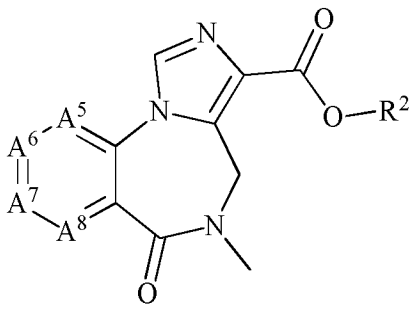


Compound 2



Compound 3

In another aspect, the present invention provides a precursor compound suitable for obtaining an ^{18}F-labelled compound of the invention wherein said precursor compound is of Formula II:



II

wherein one of A^{5-8} is a nitrogen heteroatom, one of A^{5-8} is C-LG wherein LG is a leaving group, and the remaining two of A^{5-8} are CH; and,

R^2 is as defined above for R^1 of Formula I.

The term "precursor compound" refers to a non-radioactive derivative of the ^{18}F-labelled compound of the invention, designed so that chemical reaction with a convenient chemical form of ^{18}F occurs site-specifically; can be conducted in the minimum number of steps (ideally a single step); and without the need for significant purification, to give the desired ^{18}F-labelled compound of the invention. Such precursor compounds are synthetic and can conveniently be obtained in good chemical purity.

The term "leaving group" refers to a substituent of the precursor compound as defined above which is replaced with ^{18}F when a precursor compound of the invention is reacted with a suitable source of [^{18}F]fluoride, thereby permitting incorporation of ^{18}F site-specifically to result in an ^{18}F-labelled compound of Formula I.

A preferred leaving group LG is selected from the group consisting of halogen, nitro, tri-C_{1-3} alkyl ammonium and -I^+-Ar, wherein Ar is phenyl substituted with one or more R^* groups, wherein R^* is selected from hydrogen, nitro, cyano, halogen, C_{1-10} hydroxyalkyl, C_{2-10} carboxyalkyl, C_{1-10} alkyl, C_{2-10} alkoxyalkyl, C_{1-10} hydroxyalkyl, C_{1-10} aminoalkyl, C_{1-10} haloalkyl, C_{6-14} aryl, C_{3-12} heteroaryl, C_{3-20} alkylaryl, C_{2-10} alkenyl, and C_{2-10} alkynyl.

The term "alkyl" used either alone or as part of another group is defined as any straight, branched or cyclic, saturated or unsaturated C_nH_{2n+1} group.

The term "aryl" used either alone or as part of another group is defined as any C_{6-14} molecular fragment or group which is derived from a monocyclic or polycyclic aromatic hydrocarbon, or a monocyclic or polycyclic heteroaromatic hydrocarbon.

The term "halogen" means a group selected from fluorine, chlorine, bromine, and

iodine.

The term "nitro" refers to the group $-NO_2$.

The term "cyano" refers to the group –CN.

The term "carboxyalkyl" refers to an alkyl group as defined above substituted with at least one –COOH.

The term "alkoxyalkyl" refers to an alkyl ether radical wherein the term alkyl is as defined above.

The term "hydroxyalkyl" refers to an alkyl radical as defined above wherein at least one hydrogen atom has been replaced by an -OH group.

The term "aminoalkyl" refers to an alkyl radical as defined above wherein at least one hydrogen atom has been replaced by an $-NH_2$ group.

The term "haloalkyl" refers to an alkyl radical as defined above wherein at least one hydrogen atom has been replaced by a halogen wherein halogen is as defined herein.

The term "heteroaryl" refers to an aryl as defined above wherein at least one carbon atom is replaced with a heteroatom selected from O, N and S.

The term "alkylaryl" refers to an alkyl radical as defined above in which at least one hydrogen atom is replaced by an aryl radical as defined above.

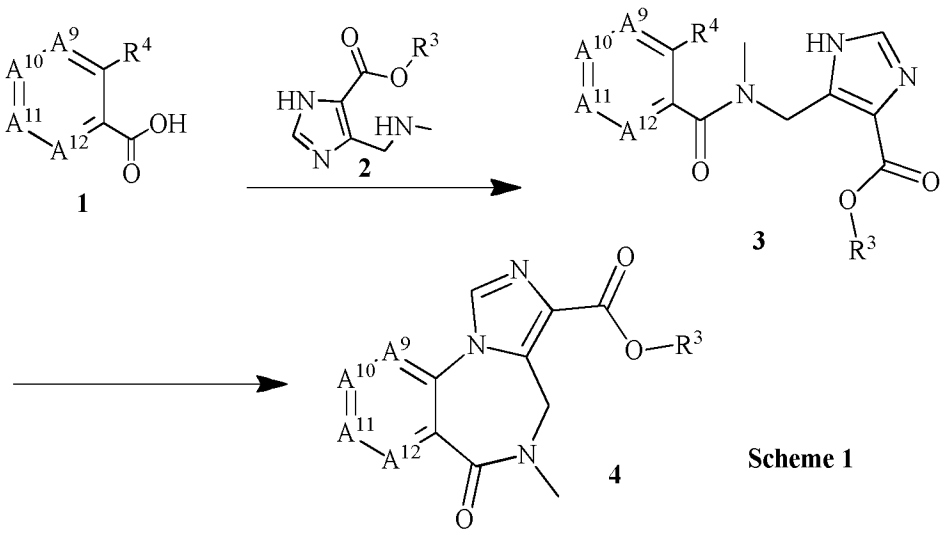
The term "heterocycle" refers herein to an aliphatic or aromatic cyclic radical wherein the cycle comprises one or more heteroatoms selected from nitrogen, oxygen or sulfur.

The term "alkenyl" means a straight-chain or branched-chain hydrocarbon radical having one or more double bonds.

The term "alkynyl" means a straight-chain or branched chain hydrocarbon radical having one or more triple bonds.

In a preferred embodiment of theprecursor compound of the invention LG is nitro or is a halogen selected from fluoro, chloro and bromo. Most preferably LG is nitro, bromo or chloro.

A precursor compound of Formula II as defined herein may be obtained using the following general reaction scheme:



Scheme 1

In Scheme 1 above, A^9-A^{12} and R^3 are as suitably and preferably defined herein for A^5-A^8 and R^2 of Formula II, respectively and R^4 is a halogen, preferably fluoro or chloro. The reaction of starting materials **1** and **2** may be effected by any standard amidation method. The carboxylic acid **1** can either be obtained commercially or can be synthesised according to the method set out in Schlosser *et al* (2005 J Org Chem; 70: 2494-2502); **2** can be prepared following the methods described in GB 2249094. Suitably **1** is converted to the corresponding activated acid, for example to an acid chloride by reaction with oxalyl chloride or to an acid imidazole with N,N'-carbonyldiimidazole. To the resulting suspension is added the imidazole carboxylate **2**. Cyclisation of the resultant **3** results in **4**, which is a precursor compound of Formula II as defined herein. Cyclisation of **3** may be effected using a suitable base, such as a alkali hydride for example sodium hydride or lithium hydride, or alkali carbonates such as cesium carbonate or potassium carbonate.

In a further aspect, the present invention provides a method to obtain an ^{18}F-labelled compound of Formula I as defined herein wherein said method comprises reaction of a precursor compound of Formula II as defined herein with a suitable source of [^{18}F]fluoride. [^{18}F]fluoride ($^{18}F^-$) for radiofluorination reactions 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. A suitable cationic counterion for this purpose should possess sufficient solubility within the anhydrous reaction solvent to maintain the solubility of $^{18}F^-$. Suitable counterions include large but soft metal ions such as rubidium or caesium, potassium complexed

with a cryptand such as Kryptofix™, or tetraalkylammonium salts. A preferred suitable source of [^{18}F]fluoride is selected from [^{18}F]potassium fluoride and [^{18}F]caesium fluoride, most preferably [^{18}F]potassium fluoride wherein Kryptofix™ is used to activate the [^{18}F]fluoride ion because of its good solubility in anhydrous solvents and enhanced $^{18}F^-$ reactivity. $^{18}F^-$ that has been made reactive in this way, reacted with a precursor compound of Formula II, results in an ^{18}F-labelled compound of Formula I.

The method to obtain the ^{18}F-labelled compound of the invention may also comprise:

(i) removal of excess [^{18}F]fluoride; and/or,

(ii) removal of organic solvent; and/or,

(iii) formulation of the resultant compound together with a biocompatible carrier to obtain a radiopharmaceutical composition suitable for mammalian administration.

The synthesis of ^{18}F-labelled compounds, particularly for use as PET tracers, is currently most conveniently carried out by means of an automated synthesis apparatus, e.g. Tracerlab™ and FASTlab™ (both GE Healthcare). FASTlab™ represents the state of the art in automated PET radiotracer synthesis platforms, so that it is desirable in the development of a new PET radiotracer that its synthesis is compatible with FASTlab™. In a preferred embodiment, the method to obtain the ^{18}F-labelled compound of the invention is automated, preferably *via* an automated synthesis apparatus. The radiochemistry is performed on the automated synthesis apparatus by fitting a "cassette" to the apparatus. Such a 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 further aspect of the present invention there is provided a cassette for carrying out the automated method of the invention comprising:

(i) a vessel containing the precursor compound of Formula II as defined herein; and

(ii) means for eluting the vessel with a suitable source of [^{18}F]fluoride as defined herein; and optionally,

(iii) an ion-exchange cartridge for removal of excess [^{18}F]fluoride.

The reagents, solvents and other consumables required for the automated 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.

Also provided by the present invention is a "radiopharmaceutical composition", which comprises the ^{18}F-labelled compound as defined herein together with a biocompatible carrier in a form suitable for mammalian administration.

The "biocompatible carrier" is a fluid, especially a liquid, in which the ^{18}F-labelled compound 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 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 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 is pyrogen-free water for injection, isotonic saline or an aqueous ethanol solution. The pH of the biocompatible carrier for intravenous injection is suitably in the range 4.0 to 10.5.

Suitable and preferred embodiments of the ^{18}F-labelled compound when comprised in the radiopharmaceutical composition of the invention are as already described herein.

The radiopharmaceutical composition may be administered parenterally, i.e. by injection, and is most preferably an aqueous solution. Such a composition may optionally contain further ingredients such as buffers; pharmaceutically acceptable solubilisers (e.g. cyclodextrins or surfactants such as Pluronic, Tween or phospholipids); pharmaceutically acceptable stabilisers or antioxidants (such as ascorbic acid, gentisic acid, ethanol or *para*-aminobenzoic acid). Where the ^{18}F-labelled compound of the invention is provided as a radiopharmaceutical composition, the method for preparation of said ^{18}F-labelled compound may further comprise the

steps required to obtain a radiopharmaceutical composition, e.g. removal of organic solvent, addition of a biocompatible buffer and any optional further ingredients. For parenteral administration, steps to ensure that the radiopharmaceutical composition is sterile and apyrogenic also need to be taken.

The present invention provides in a further aspect the ^{18}F-labelled compound as suitably and preferably defined herein for use in a method of *in vivo* imaging. Most preferably the ^{18}F-labelled compound for use in a method of *in vivo* imaging is provided as the radiopharmaceutical composition as suitably and preferably defined herein. The *in vivo* imaging method of the invention comprises the following steps:

(i) administering to a subject the ^{18}F-labelled compound of Formula I as defined herein;

(ii) allowing said administered ^{18}F-labelled compound to bind specifically to $GABA_A$ receptors in said subject;

(iii) detecting signals derived from the positron emission decay of ^{18}F present in said specifically bound ^{18}F-labelled compound; and,

(iv) generating an image of the location and amount of said signals, wherein said signals represent the distribution of $GABA_A$ receptors in said subject.

The step of "administering" the ^{18}F-labelled compound is preferably carried out parenterally, and most preferably intravenously. The intravenous route represents the most efficient way to deliver the ^{18}F-labelled compound throughout the body of the subject, and therefore also across the blood-brain barrier (BBB) and into contact with $GABA_A$ receptors expressed in the CNS of said subject. The ^{18}F-labelled compound of the invention is preferably administered as the radiopharmaceutical composition of the invention, as defined herein. Alternatively, the *in vivo* imaging method of the invention can be understood to start at step (ii) wherein said ^{18}F-labelled compound of Formula I has been pre-administered to said subject.

Following the administering step and preceding the detecting step, the ^{18}F-labelled compound is allowed to bind to $GABA_A$ receptors. The ^{18}F-labelled compound moves dynamically through the subject's body, coming into contact with various tissues therein. Once the ^{18}F-labelled compound comes into contact with $GABA_A$ receptors, a

specific interaction takes place such that clearance of the ^{18}F-labelled compound from tissue with $GABA_A$ receptors takes longer than from tissue without, or having less $GABA_A$ receptors. A certain point in time is reached when detection of ^{18}F-labelled compound specifically bound to $GABA_A$ receptors is enabled as a result of the ratio between ^{18}F-labelled compound bound to tissue with $GABA_A$ receptors versus that bound in tissue without, or having less $GABA_A$ receptors.

The "detecting" step of the method of the invention involves detection of signals derived from the positron emission decay of ^{18}F by means of a detector sensitive to said signals, a scintillator present in the PET scanner. In positron-emission decay, which is also known as positive beta decay, a positron is emitted, and then travels up to a few millimetres until it encounters an electron. The encounter of the positron and the electron results in the production of a pair of annihilation (gamma) photons that are emitted at around 180 degrees to each other. It is these annihilation photons that are the "signals derived from the positron emission decay".

The "generating" step of the method of the invention is carried out by a computer which applies a reconstruction algorithm to the acquired signal data to yield a dataset. This dataset is then manipulated to generate an image showing the location and/or amount of signals emitted by ^{18}F.

The "subject" of the invention can be any human or animal subject. Preferably the subject of the invention is a mammal. Most preferably, said subject is an intact mammalian body *in vivo*. In an especially preferred embodiment, the subject of the invention is a human.

The *in vivo* imaging method may be used to study $GABA_A$ receptors in healthy subjects, or in subjects known or suspected to have a pathological condition associated with abnormal expression of $GABA_A$ receptors (a "$GABA_A$ condition"). Examples of such $GABA_A$ conditions where the *in vivo* imaging method of the invention would be of use include epilepsy, anxiety disorders, Parkinson's disease and chronic pain. The ^{18}F-labelled compound of the invention is particularly suited to imaging $GABA_A$ receptor expression in the central nervous system (CNS) of a subject. In a preferred embodiment, the *in vivo* imaging method of the invention is carried out on a subject who is known or is suspected to have a $GABA_A$ condition. The *in vivo* imaging method of the invention is therefore a useful tool in the diagnosis of $GABA_A$-related

pathological conditions. Accordingly, in another aspect, the present invention provides a diagnostic method comprising the *in vivo* imaging method as defined above and the additional step of:

(v) attributing the distribution of $GABA_A$ receptors in said subject to a particular diagnosis.

In an alternative embodiment, the *in vivo* imaging method of the invention may be employed in selecting the most appropriate treatment for a condition. In addition, the *in vivo* imaging method may be carried out repeatedly during the course of a treatment regimen for said subject, said treatment regimen comprising administration of a drug to combat a $GABA_A$ condition. For example, the *in vivo* imaging method as suitably and preferably defined herein can be carried out before, during and after treatment with a drug to combat a $GABA_A$ condition. In this way, the effect of said treatment can be monitored over time. Positron emission tomography (PET), which is the imaging method used wherein the radioisotope is ^{18}F, has excellent sensitivity and resolution, so that even relatively small changes in a lesion can be observed over time, which is advantageous for treatment monitoring. PET scanners routinely measure radioactivity concentrations in the picomolar range. Micro-PET scanners now approach a spatial resolution of about 1mm, and clinical scanners about 4-5mm.

In another aspect, the present invention provides the ^{18}F-labelled compound as suitably and preferably defined herein for use in any one of the methods of *in vivo* imaging, diagnosis and treatment monitoring as defined above.

In a yet further aspect, the present invention provides the ^{18}F-labelled compound as defined herein for use in the manufacture of a radiopharmaceutical composition as defined herein for use in any one of the methods of *in vivo* imaging, diagnosis and treatment monitoring as defined above.

The invention is now illustrated by a series of non-limiting examples.

Brief Description of the Examples

Example 1 describes the synthesis of ethyl 8-fluoro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[2,3-f][1,4]diazepine-3-carboxylate, a non-radioactive version of the ^{18}F-labelled compound of the invention referred to above as Compound 1.

Example 2 describes the synthesis of tert-butyl 7-fluoro-5-methyl-6-oxo-5,6-dihydro-

4H-imidazo[1,5-a]pyrido[3,4-f][1,4]diazepine-3-carboxylate and tert-butyl 4-fluoro-6-methyl-5-oxo-6,7-dihydro-5H-imidazo[1,5-a]pyrido[3,2-f][1,4]diazepine-8-carboxylate, two non-radioactive versions of ^{18}F-labelled compounds of the invention referred to hereinabove as Compounds 2 and 3.

Example 3 describes the synthesis of tert-butyl 7-chloro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[3,4-f][1,4]diazepine-3-carboxylate and tert-butyl 4-chloro-6-methyl-5-oxo-6,7-dihydro-5H-imidazo[1,5-a]pyrido[3,2-f][1,4]diazepine-8-carboxylate, two precursor compounds of the invention suitable for the preparation of Compounds 2 and 3 of the invention.

Example 4 describes the synthesis of ethyl 8-chloro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[2,3-f][1,4]diazepine-3-carboxylate, a precursor compound of the invention suitable for obtaining Compound 1 of the invention.

Example 5 describes the *in vitro* assay used to evaluate the affinity of the compounds of the invention.

Example 6 describes the general procedure used for radiolabelling to obtain ^{18}F compounds of the present invention.

Example 7 describes the radiosynthesis of [^{18}F]ethyl-8-fluoro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[2,3-f]azepine-3-carboxylate, Compound 1 of the invention.

Example 8 describes the radiosynthesis of [^{18}F]tert-butyl-7-fluoro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[3,4-f]azepine-3-carboxylate, Compound 2 of the invention.

Example 9 describes the radiosynthesis of [^{18}F]tert-butyl-4-fluoro-6-methyl-5-oxo-6,7-dihydro-5H-imidazo[1,5-a]pyrido[3,2-f]azepine-8-carboxylate, Compound 3 of the invention.

List of Abbreviations used in the Examples

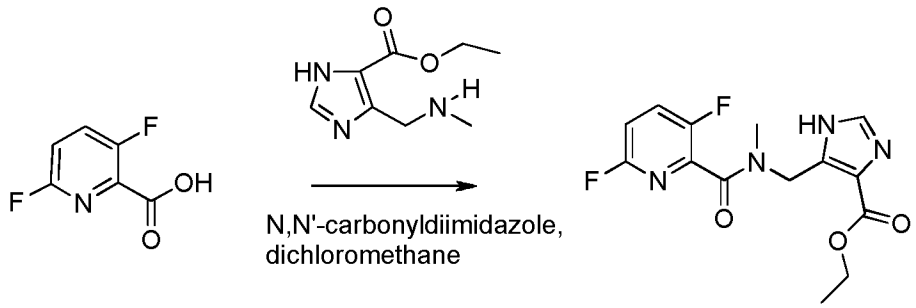
HPLC	high performance liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
NMR	nuclear magnetic resonance

RT room temperature

Examples

Example 1: Synthesis of ethyl 8-fluoro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[2,3-f][1,4]diazepine-3-carboxylate

Example 1(i): ethyl 5-((3,6-difluoro-N-methylpicolinamido)methyl)-1H-imidazole-4-carboxylate

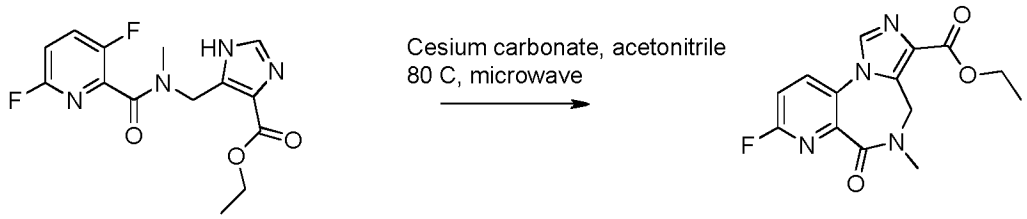


3,6-difluoropyridine-2-carboxylic acid (Fluorochem, 276 mg, 1.7 mmol) and N,N'-carbonyldiimidazole (300 mg, 1.9 mmol) in dichloromethane (10 mL) were stirred at room temperature for 2 h, under an atmosphere of nitrogen. To the resulting suspension was added ethyl 4-((methylamino)methyl)-1H-imidazole-5-carboxylate (378 mg, 2.1 mmol), prepared as described in GB 2249094. The mixture was stirred at room temperature for 41 h. The resulting suspension was washed with saturated sodium bicarbonate (25 mL). The organic phase was collected, passed through a phase separator and concentrated *in vacuo* to afford 416 mg of brown oil. The crude material was purified by column chromatography with silica eluting dichloromethane (A) and methanol (B) (0% → 5% B, 50 g, 19.5 CV, 40 mL/min, Flashmaster Companion) to afford 123 mg (23%) of ethyl 5-((3,6-difluoro-N-methylpicolinamido)methyl)-1H-imidazole-4-carboxylate as a glass.

^{1}H NMR (300 MHz, CD_3OD): δ_H 1.31 (1.5H, *t*, *J* = 7.1 Hz, $OCH_2C\underline{H}_3$), 1.37 (1.5H, *t*, *J* = 7.1 Hz, $OCH_2C\underline{H}_3$), 2.92 (1.5H, *s*, $NC\underline{H}_3$), 3.19 (1.5H, *s*, $NC\underline{H}_3$), 4.23 (1H, *q*, *J* = 7.1 Hz, $OC\underline{H}_2CH_3$), 4.36 (1H, *q*, *J* = 7.1 Hz, $OC\underline{H}_2CH_3$), 4.83 (1H, *s*, $NC\underline{H}_2$), 5.07 (1H, *s*, $NC\underline{H}_2$), 7.18 (0.5H, *dt*, *J* = 8.9, 3.0 Hz, NCFC$\underline{H}$CH), 7.23 (0.5H, *dt*, *J* = 8.9, 3.0 Hz, NCFC$\underline{H}$CH), 7.73 (0.5H, *s*, NC$\underline{H}$N), 7.77 (0.5H, *s*, NC$\underline{H}$N), and 7.80-7.93 (1H, *m*, NCFCHC$\underline{H}$).

LC-MS: *m/z* calcd for $C_{14}H_{14}F_2N_4O_3$ 324.1; found, 325.1 $(M+H)^+$, and 322.9 $(M-H)^+$.

Example 1(ii): ethyl 8-fluoro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[2,3-f][1,4]diazepine-3-carboxylate



To a mixture of ethyl 5-((3,6-difluoro-N-methylpicolinamido)methyl)-1H-imidazole-4-carboxylate (43 mg, 0.013 mmol) in acetonitrile (6 mL) was added cesium carbonate (65 mg, 0.020 mmol). The reaction mixture was stirred at room temperature for 30 mins. The reaction mixture was heated to 80 C in a sealed tube in the microwave for 7.5 h. The reaction mixture was concentrated *in vacuo*. The resulting residue was dissolved in dichloromethane (6 mL) and washed with water (6 mL). The aqueous layer was separated and back extracted with dichloromethane (4 mL). The organic phases were combined, passed through a phase separator and concentrated *in vacuo*. The crude product (34 mg) was purified by column chromatography with silica eluting dichloromethane (A) and methanol (B) (0-100% B, 10g, 17 CV, 40 mL/min, Combiflash Companion) to afford 5 mg of material (77% pure by HPLC). The material was purified by column chromatography with silica eluting dichloromethane (A) and methanol (B) (0-5% B, 4 g, 57.7 CV, 18 mL/min, Combiflash Companion) to afford 1.4 mg (4%) of ethyl 8-fluoro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[2,3-f][1,4]diazepine-3-carboxylate as a glass.

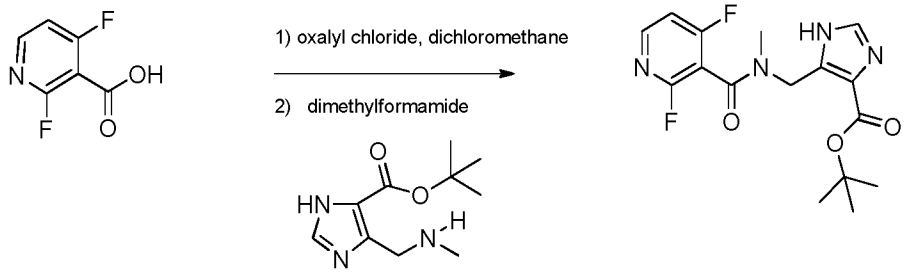
^{1}H NMR (300 MHz, CD_3CN): δ_H 1.37 (3H, *t*, *J* = 7.1 Hz, $OCH_2C\underline{H}_3$), 3.14 (3H, *s*, $NC\underline{H}_3$), 4.33-4.37 (2H, *s*, $OC\underline{H}_2CH_3$), 4.46 (1H, *d*, *J* = 15.3 Hz, $NC\underline{H}_2$), 5.09 (1H, *d*, *J* = 16.5 Hz, $NC\underline{H}_2$), 7.36 (1H, *dd*, *J* = 8.9, 6.8 Hz, NCFC<u>H</u>CH), 8.00 (1H, *s*, NC<u>H</u>N), and 8.15 (1H, *dd*, *J* = 8.9, 3.6 Hz, NCFCHC<u>H</u>).

^{19}F NMR (283 MHz, CD_3CN): δ_F -68.6

LC-MS: *m/z* calcd for $C_{14}H_{13}FN_4O_3$ 304.1; found, 305.2 $(M+H)^+$.

Example 2: Synthesis of tert-butyl 7-fluoro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[3,4-f][1,4]diazepine-3-carboxylate and tert-butyl 4-fluoro-6-methyl-5-oxo-6,7-dihydro-5H-imidazo[1,5-a]pyrido[3,2-f][1,4]diazepine-8-carboxylate

Example 2(i): tert-butyl 5-((2,4-difluoro-N-methylnicotinamido)methyl)-1H-imidazole-4-carboxylate

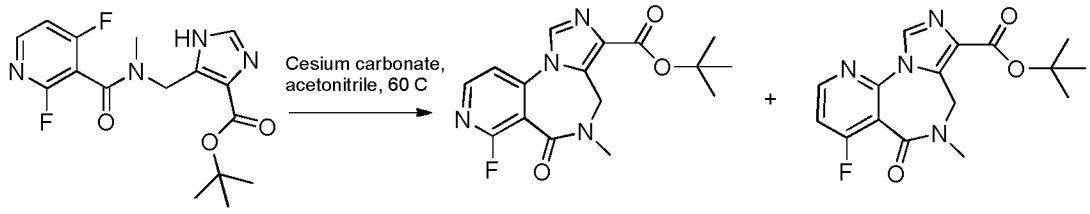


To a suspension of 2,4-difluoropyridine-3-carboxylic acid (509 mg, 3.2 mmol), prepared as described by Schlosser *et al* (2005 J Org Chem; 70: 2494-2502), in anhydrous dichloromethane (2 mL) at O C under nitrogen was added oxalyl chloride (1.62 g, 12.8 mmol, 1.12 mL) and anhydrous dimethylformamide (1 drop). The mixture was stirred at RT for 0.5 h. The solvents were removed *in vacuo* and the residue dissolved in anhydrous dimethylformamide (2 mL). *Tert*-butyl 4-((methylamino)methyl)-1H-imidazole-5-carboxylate (676 mg, 3.2 mmol), prepared as described in GB 2249094 was added and the mixture stirred at RT for 24 h. The solvents were removed *in vacuo*, the residue quenched with water (20 mL), extracted with dichloromethane (3 x 20 mL), dried (magnesium sulphate) and solvents removed *in vacuo*. The crude material was purified by silica gel chromatography eluting with dichloromethane (A) and methanol (B) (10% (B), 80 g, 2.0 CV, 60 mL/min) to afford 421 mg (37%) of *tert*-butyl 5-((2,4-difluoro-N-methylnicotinamido)methyl)-1H-imidazole-4-carboxylate as a colourless foam. The compound was found to be rotameric by ^{1}H NMR.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.51 (2H, *s*, C<u>H</u>$_3$ x 3), 1.61 (7H, *s*, C<u>H</u>$_3$ x 3), 3.02 (2H, *s*, NC<u>H</u>$_3$), 3.22 (1H, *s*, NC<u>H</u>$_3$), 4.35-4.73 (0.5H, *m*, NC<u>H</u>$_2$), 5.07 (1.5H, *s*, NC<u>H</u>$_2$), 6.96-7.08 (1H, *m*, FCNCHC<u>H</u>), 7.61 (0.3H, *s*, NC<u>H</u>N), 7.63 (0.7H, *s*, NC<u>H</u>N), and 8.16-8.30 (1H, *m*, FCNC<u>H</u>CH).

LC-MS: *m/z* calcd for $C_{16}H_{18}F_2N_4O_3$ 352.1; found, 353.1 $(M+H)^+$.

Example 2(ii): tert-butyl 7-fluoro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[3,4-f][1,4]diazepine-3-carboxylate and tert-butyl 4-fluoro-6-methyl-5-oxo-6,7-dihydro-5H-imidazo[1,5-a]pyrido[3,2-f][1,4]diazepine-8-carboxylate



To a solution of *tert*-butyl 5-((2,4-difluoro-N-methylnicotinamido)methyl)-1H-imidazole-4-carboxylate (35 mg, 0.1 mmol) in anhydrous acetonitrile (1 mL) under nitrogen was added cesium carbonate (33 mg, 0.1 mmol). The mixture was stirred at 60 C for 1 h. The solvents were removed *in vacuo*, the residue quenched with water (1 mL), extracted with dichloromethane (3 x 5 mL), dried (magnesium sulphate) and solvents removed *in vacuo*. The crude material was purified by silica gel chromatography eluting with dichloromethane (A) and methanol (B) (5% (B), 40 g, 2.5 CV, 40 mL/min) to afford 2 mg (10%) of *tert*-butyl 4-fluoro-6-methyl-5-oxo-6,7-dihydro-5H-imidazo[1,5-a]pyrido[3,2-f][1,4]diazepine-8-carboxylate as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.64 (9H, *s*, C<u>H</u>$_3$ x 3), 3.24 (3H, *s*, NC<u>H</u>$_3$), 4.40 (1H, *br d*, *J* = 16.0 Hz, NC<u>H</u>$_2$), 5.24 (1H, *br d*, *J* = 16.0 Hz, NC<u>H</u>$_2$), 7.21 (1H, *dd*, J_{HF} = 8.6 Hz, J_{HH} = 5.5 Hz, FCC<u>H</u>CHN), 8.30 (1H, *s*, NC<u>H</u>N), and 8.57 (1H, *dd*, J_{HF} = 7.3 Hz, J_{HH} = 5.5 Hz, FCCHC<u>H</u>N).

^{19}F NMR (283 MHz, $CDCl_3$): δ_F –95.3

LC-MS: *m/z* calcd for $C_{16}H_{17}FN_4O_3$ 332.1; found, 333.2 $(M+H)^+$.

Further elution with dichloromethane (A) and methanol (B) (5% (B), 40 g, 3.5 CV, 40 mL/min) afforded 25 mg (80%) of *tert*-butyl 7-fluoro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[3,4-f][1,4]diazepine-3-carboxylate as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.62 (9H, *s*, C<u>H</u>$_3$ x 3), 3.21 (3H, *s*, NC<u>H</u>$_3$), 4.39 (1H, *d*, *J* = 15.9 Hz, NC<u>H</u>$_2$), 5.21 (1H, *d*, *J* = 15.9 Hz, NC<u>H</u>$_2$), 7.29 (1H, *d*, *J*= 5.5 Hz, FCNCHC<u>H</u>), 7.96 (1H, *s*, NC<u>H</u>N), and 8.40 (1H, *dd*, J_{HH} = 5.5 Hz, J_{HF} = 0.6 Hz, FCNC<u>H</u>CH).

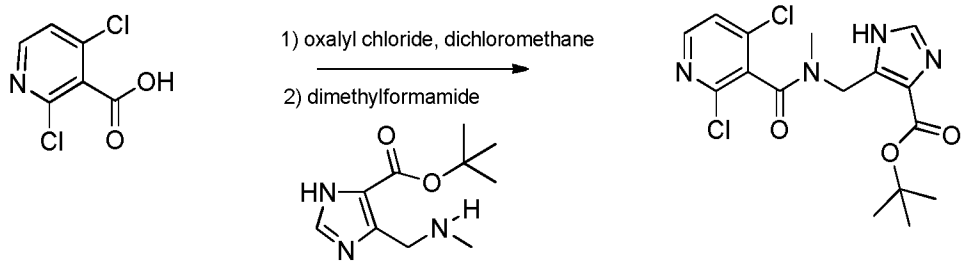
^{19}F NMR (283 MHz, $CDCl_3$): δ_F –59.9

LC-MS: *m/z* calcd for $C_{16}H_{17}FN_4O_3$ 332.1; found, 333.1 $(M+H)^+$.

<u>Example 3: Synthesis of tert-butyl 7-chloro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[3,4-f][1,4]diazepine-3-carboxylate and tert-butyl 4-chloro-6-</u>

methyl-5-oxo-6,7-dihydro-5H-imidazo[1,5-a]pyrido[3,2-f][1,4]diazepine-8-carboxylate

Example 3(i): tert-butyl 5-((2,4-dichloro-N-methylnicotinamido)methyl)-1H-imidazole-4-carboxylate

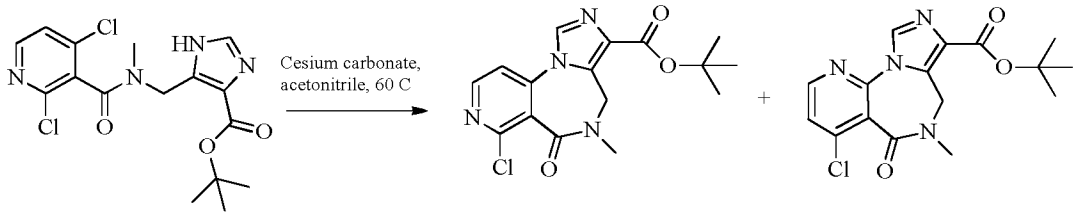


To a suspension of 2,4-dichloropyridine-3-carboxylic acid (Aldrich, 611 mg, 3.2 mmol) in anhydrous dichloromethane (2 mL) at O C under nitrogen was added oxalyl chloride (1.62 g, 12.8 mmol, 1.12 mL) and anhydrous dimethylformamide (1 drop). The mixture was stirred at RT for 0.5 h. The solvents were removed *in vacuo* and the residue dissolved in anhydrous dimethylformamide (1.0 mL). *Tert*-butyl 4-((methylamino)methyl)-1H-imidazole-5-carboxylate (676 mg, 3.2 mmol), prepared as described in GB 2249094 was added and the mixture stirred at RT for 24 h. The solvents were removed *in vacuo*, the residue quenched with water (20 mL), extracted with dichloromethane (3 x 20 mL), dried (magnesium sulphate) and solvents removed *in vacuo*. The crude material was purified by silica gel chromatography eluting with dichloromethane (A) and methanol (B) (5% (B), 80 g, 4.0 CV, 60 mL/min) to afford 342 mg (28%) of *tert*-butyl 5-((2,4-dichloro-N-methylnicotinamido)methyl)-1H-imidazole-4-carboxylate as a colourless oil. The compound was found to be rotameric by ^{1}H NMR.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.49 (3H, *s*, CH_3 x 3), 1.62 (6H, *s*, CH_3 x 3), 2.97 (2.5H, *s*, NCH_3), 3.34 (0.5H, *s*, NCH_3), 4.61 and 4.69 (0.5H, 2 x *d*, *J*= 16.0 Hz, NCH_2), 5.11 (1.5H, *s*, NCH_2), 7.27-7.37 (1H, *m*, ClCNCHCH), 7.67 (0.3H, *s*, NCHN), 7.73 (0.7H, *s*, NCHN), and 8.25-8.37 (1H, *m*, ClCNCHCH).

LC-MS: *m/z* calcd for $C_{16}H_{18}Cl_2N_4O_3$ 384.1; found, 384.9 $(M+H)^+$.

Example 3(ii): tert-butyl 7-chloro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[3,4-f][1,4]diazepine-3-carboxylate and tert-butyl 4-chloro-6-methyl-5-oxo-6,7-dihydro-5H-imidazo[1,5-a]pyrido[3,2-f][1,4]diazepine-8-carboxylate



To a solution of *tert*-butyl 5-((2,4-dichloro-N-methylnicotinamido)methyl)-1H-imidazole-4-carboxylate (342 mg, 0.98 mmol) in anhydrous acetonitrile (7 mL) under nitrogen was added cesium carbonate (319 mg, 0.98 mmol). The mixture was stirred at 60 C for 2 h. The solvents were removed *in vacuo*, the residue quenched with water (10 mL), extracted with dichloromethane (3 x 10 mL), dried (magnesium sulphate) and solvents removed *in vacuo*. The crude material was purified by silica gel chromatography eluting with dichloromethane (A) and methanol (B) (5% (B), 80 g, 7.0 CV, 60 mL/min) to afford impure product (219 mg). The sample was repurified by silica gel chromatography eluting with dichloromethane (A) and methanol (B) (5% (B), 80 g, 1.5 CV, 60 mL/min) to afford 44 mg (13%) of *tert*-butyl 4-chloro-6-methyl-5-oxo-6,7-dihydro-5H-imidazo[1,5-a]pyrido[3,2-f][1,4]diazepine-8-carboxylate as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.64 (9H, *s*, C<u>H</u>$_3$ x 3), 3.23 (3H, *s*, NC<u>H</u>$_3$), 4.41 (1H, *d*, *J*= 16.3 Hz, NC<u>H</u>$_2$), 5.23 (1H, *d*, *J*= 16.3 Hz, NC<u>H</u>$_2$), 7.50 (1H, *d*, *J*= 5.2 Hz, ClCC<u>H</u>CH<u>N</u>), 8.28 (1H, *s*, NC<u>H</u>N), and 8.46 (1H, *d*, *J*= 5.2 Hz, ClCCHC<u>H</u><u>N</u>).

^{13}C NMR (75 MHz; $CDCl_3$): δ_C 28.2, 34.8, 42.2, 82.1, 122.6, 125.5, 130.7, 133.6, 135.8, 144.8, 147.1, 150.6, 161.9, and 162.2.

LC-MS: *m/z* calcd for $C_{16}H_{17}ClN_4O_3$ 348.1; found, 349.0 $(M+H)^+$.

Further elution with dichloromethane (A) and methanol (B) (5% (B), 80 g, 2.2 CV, 60 mL/min) afforded 141 mg (41%) of *tert*-butyl 7-chloro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[3,4-f][1,4]diazepine-3-carboxylate as a white solid.

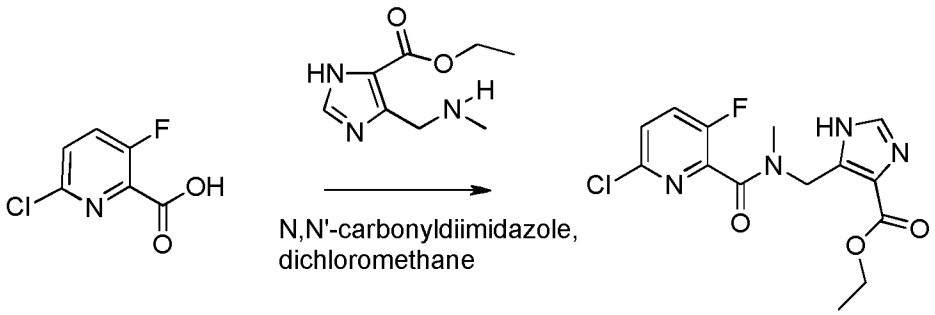
^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.64 (9H, *s*, C<u>H</u>$_3$ x 3), 3.22 (3H, *s*, NC<u>H</u>$_3$), 4.37 (1H, *d*, *J*= 15.9 Hz, NC<u>H</u>$_2$), 5.20 (1H, *d*, *J*= 15.9 Hz, NC<u>H</u>$_2$), 7.29 (1H, *d*, *J*= 5.3 Hz, ClCNCHC<u>H</u>), 7.94 (1H, *s*, NC<u>H</u>N), and 8.56 (1H, *d*, *J*= 5.3 Hz, ClCNC<u>H</u>CH).

^{13}C NMR (75 MHz; $CDCl_3$): δ_C 28.3, 34.9, 42.2, 82.5, 114.9, 123.7, 131.5, 134.4, 134.5, 140.5, 151.2, 153.0, 161.5, and 162.6.

LC-MS: *m/z* calcd for $C_{16}H_{17}ClN_4O_3$ 348.1; found, 349.0 $(M+H)^+$.

Example 4: Synthesis of ethyl 8-chloro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[2,3-f][1,4]diazepine-3-carboxylate

Example 4(i): ethyl 5-((6-chloro-3-fluoro-N-methylpicolinamido)methyl)-1H-imidazole-4-Carboxylate

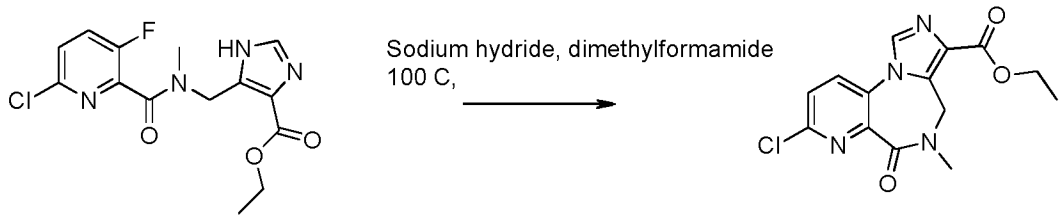


To a suspension of 2-chloro-5-fluoropyridine-6-carboxylic acid (Asymchem, 450 mg, 2.6 mmol) in dichloromethane (50 mL) was added N,N'-carbonyldiimidazole (468 mg, 2.9 mmol) with stirring, under an atmosphere of nitrogen. After 1 h stirring at RT, ethyl 4-((methylamino)methyl)-1H-imidazole-5-carboxylate (571 mg, 3.1 mmol), prepared as described in GB2249094, was added. The mixture was stirred for 2 h at RT. The reaction mixture was diluted with dichloromethane (25 mL) and washed with saturated sodium bicarbonate solution (25 mL). The organic layer was collected through a phase separator then concentrated *in vacuo*. The crude product (830 mg) was purified by column chromatography with silica eluting dichloromethane (A) and methanol (B) (0-3% B, 100g, 17 CV, 60 mL/min, Flashmaster Companion) to afford 432 mg (49%) of ethyl 5-((6-chloro-3-fluoro-N-methylpicolinamido)methyl)-1H-imidazole-4-carboxylate as a mixture of rotamers.

^{1}H NMR (300 MHz, CD_3OD): d_H 1.24-1.36 (3H, *m*, $OCH_2C\underline{H}_3$), 2.85-3.15 (3H, *m*, $NC\underline{H}_3$), 4.14-4.34 (2H, *m*, $OC\underline{H}_2CH_3$), 4.65-5.00 (2H, *m*, $NC\underline{H}_2$), and 7.59-7.66 (3H, *m*, ClCC<u>H</u>CHCF, ClCCHC<u>H</u>CF, and NC<u>H</u>N).

LC-MS: m/z calcd for $C_{14}H_{14}ClFN_4O_3$ 340.1; found 340.9 $(M+H)^+$, and 339.2 $(M-H)^-$.

Example 4(ii): ethyl 8-chloro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[2,3-f][1,4]diazepine-3-carboxylate



To a mixture of ethyl 5-((6-chloro-3-fluoro-N-methylpicolinamido)methyl)-1H-imidazole-4-carboxylate (355 mg, 1.0 mmol) in dimethylformamide (1 mL) was added sodium hydride (40 mg of a 60% dispersion in mineral oil, 1.0 mmol). The reaction mixture was stirred at RT, under an atmosphere of nitrogen, for 0.5 h. The reaction mixture was heated to 100°C overnight. The volatiles were removed *in vacuo*. The reaction mixture was diluted with a 50:50 mixture of brine/water (25 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried over magnesium sulfate. The magnesium sulfate was removed by filtration and the filtrate was concentrated *in vacuo* to afford a white foam. The crude product (270 mg) was purified by column chromatography with silica eluting dichloromethane (A) and methanol (B) spiked with a few drops of ammonia (0-5% B, 100g, 25 CV, 60 mL/min, Flashmaster Companion) to afford 74 mg (23%) of ethyl 8-chloro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[2,3-f][1,4]diazepine-3-carboxylate as a white solid.

^{1}H NMR (300 MHz, CD_3OD): d_H 1.37 (3H, *t*, *J* = 7.0 Hz, $OCH_2C\underline{H}_3$), 3.14 (3H, *s*, $NC\underline{H}_3$), 4.33-4.48 (3H, *m*, $OC\underline{H}_2CH_3$ and $NC\underline{H}_aH_b$), 5.08 (1H, *d*, *J* = 14.7 Hz, $NCH_a\underline{H}_b$), 7.70 (1H, *d*, *J* = 8.6 Hz, ClCC<u>H</u>CH), 8.00 (1H, *s*, NC<u>H</u>N), and 8.00 (1H, *d*, *J* = 8.6 Hz, ClCCHC<u>H</u>).

LC-MS: m/z calcd for $C_{14}H_{13}ClN_4O_3$ 320.1; found 321.0 $(M+H)^+$.

Example 5: In Vitro Affinity Assay

To assess affinity of compounds of the invention, a competitive radioligand binding assay was carried out that utilised tritiated flumazenil as the competitive agent. Tritiated flumazenil was purchased from NEN Perkin Elmer (Cat. NET757250UC) at a concentration of 1mCi/mL. Briefly, 10µl of test compound was incubated with a crude homogenate of rat cerebellum in the presence of 2nM tritiated flumazenil (diluted to 40nM). Homogenate was prepared by homogenisation of cerebellum with Dounce homogenizer in 10X vol homogenization buffer (10mM KH_2PO_4 buffer pH 7.4). The

homogenate was centrifuged at 48,000g (using SW40Ti rotor=19561RPM) 30min at 4°C. The homogenate was kept on ice at all times. After 90 min the assay was filtered through a glass fibre mat, thereby filtering out the rat homogenate and the ligand bound to it. The amount of activity on the filter mat was then measured using liquid scintillation. The affinity data for non-radioactive Compound 1, along with the commercially-available prior art compound flumazenil is presented in Table 1 below:

Table 1: *In vitro* affinity data for FMZ (flumazenil) and analogues of FMZ.

	FMZ	**Compound 1**	**Compound 2**	**Compound 3**
Ki (nM)	0.5	14	3.90	1.29
LogD		7.4	7.4	7.4

Example 6: General Radiolabelling Procedure

The following general procedure was used in each of Examples 7-9.

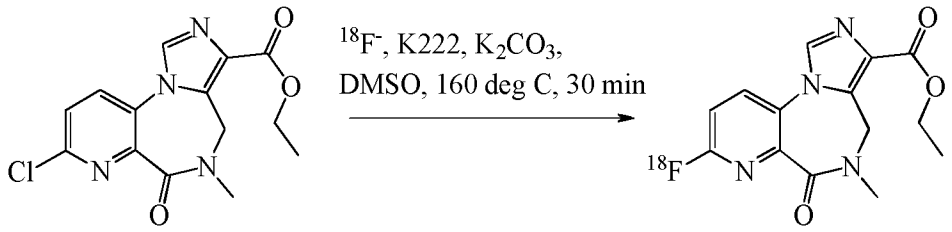
Radiolabelling was carried out on a TRACERlab FX F-N (GE Healthcare). [^{18}F]Fluoride was trapped on a QMA cartridge and then transferred to the reaction vessel using a solution of Kryptofix 2.2.2 (7.1 mg) and K_2CO_3 (3.6 mg) in MeCN (0.7 mL) and water (0.3 mL) from vial 1. The solution was dried at 100°C for 10 minutes then 120°C for 20 minutes using nitrogen plus vacuum flow and then cooled to 50°C.

To the dried [^{18}F]fluoride was added precursor compound of the invention (5 mg) in DMSO (anhydrous. 1 mL) from vial 3. The reaction mixture was heated at 160°C for 30 minutes then cooled to 50 °C. The reaction mixture was diluted with 10 mM phosphoric acid (3.5 mL) and transferred to the crude product vessel.

The crude product was then transferred onto the preparative HPLC loop using nitrogen gas pressure. Preparative HPLC was then started and to give a radioactive peak (details for specific compounds of the invention given in following examples) which was cut into a vessel containing water (15-20 mL). The mixture was trapped on a Waters tC18 light SPE (pre conditioned with 1 mL ethanol then 2 mL water). The SPE cartridge was washed with water (3 mL) and the product eluted into a P6 vial using EtOH (0.5 mL) and phosphate buffered saline (4.5 mL). The final formulation volume can be varied by

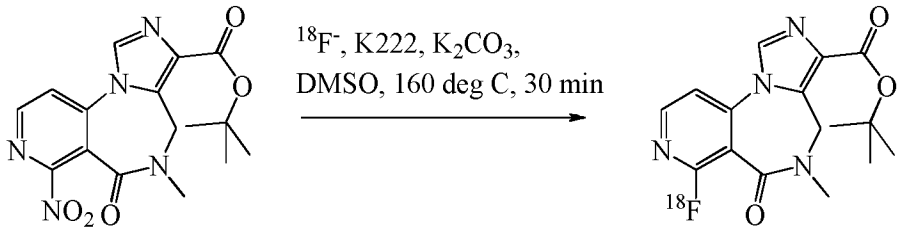
changing the volume of phosphate buffered saline used.

Example 7: Radiosynthesis of [^{18}F]ethyl-8-fluoro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[2,3-f]azepine-3-carboxylate



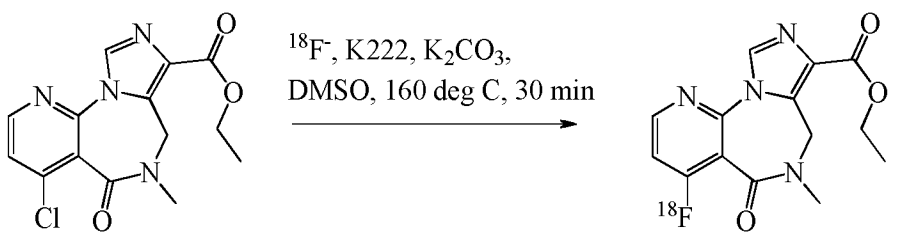
Preparative HPLC method	HPLC Column	Phenomenex Prodigy ODS-prep 250x10mm 10 µ
	Solvent	A = Water, B = MeCN, 20% B isocratic
	Flow rate	4 mL/min
	UV	254 nm
	Loop	5 mL
	Retention time	12.2 minutes
Analytical HPLC method	HPLC Column	Onyx C18 100 x 4.6 mm
	Solvent	A = Water, B = MeCN Isocratic 20% B
	Flow rate	2 mL/min
	UV	254 nm
	Loop	100 µL
	Retention time	5.2 minutes
Radiochemical yield	19% (non-decay corrected)	
Radiochemical purity	>99%	

Example 8: Radiosynthesis of [^{18}F]tert-butyl-7-fluoro-5-methyl-6-oxo-5,6-dihydro-4H-imidazo[1,5-a]pyrido[3,4-f]azepine-3-carboxylate



Preparative HPLC method	HPLC Column	Phenomenex Prodigy ODS-prep 250x10mm 10 µ
	Solvent	A = Water, B = MeCN, 30% B isocratic
	Flow rate	4 mL/min
	UV	254 nm
	Loop	5 mL
	Retention time	15.1 minutes
Analytical HPLC method	HPLC Column	Onyx C18 100 x 4.6 mm
	Solvent	A = Water, B = MeCN Isocratic 20% B
	Flow rate	2 mL/min
	UV	254 nm
	Loop	100 µL
	Retention time	10.8 minutes
Radiochemical yield	31% (non-decay corrected)	
Radiochemical purity	>99%	

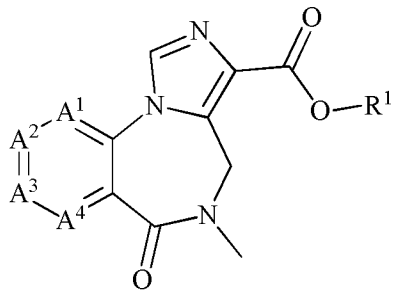
Example 9: Radiosynthesis of [^{18}F]tert-butyl-4-fluoro-6-methyl-5-oxo-6,7-dihydro-5H-imidazo[1,5-a]pyrido[3,2-f]azepine-8-carboxylate



<table>
<tr><td>Preparative HPLC method</td><td><table>
<tr><td>HPLC Column</td><td>Phenomenex Prodigy ODS-prep 250x10mm 10 µ</td></tr>
<tr><td>Solvent</td><td>A = Water, B = MeCN, 30% B isocratic</td></tr>
<tr><td>Flow rate</td><td>4 mL/min</td></tr>
<tr><td>UV</td><td>254 nm</td></tr>
<tr><td>Loop</td><td>5 mL</td></tr>
<tr><td>Retention time</td><td>25 minutes</td></tr>
</table></td></tr>
<tr><td>Analytical HPLC method</td><td><table>
<tr><td>HPLC Column</td><td>Onyx C18 100 x 4.6 mm</td></tr>
<tr><td>Solvent</td><td>A = Water, B = MeCN Isocratic 20% B</td></tr>
<tr><td>Flow rate</td><td>2 mL/min</td></tr>
<tr><td>UV</td><td>254 nm</td></tr>
<tr><td>Loop</td><td>100 µL</td></tr>
<tr><td>Retention time</td><td>11.8 minutes</td></tr>
</table></td></tr>
<tr><td>Radiochemical yield</td><td>14% (non-decay corrected)</td></tr>
<tr><td>Radiochemical purity</td><td>>99%</td></tr>
</table>

Claims

(1) An ^{18}F-labelled compound of Formula I:



I

wherein one of A^1-A^4 is a nitrogen heteroatom, one of A^1-A^4 is C-^{18}F and the remaining two of A^1-A^4 are CH; and,

R^1 is a straight or branched C_{1-4} alkyl.

(2) The ^{18}F-labelled compound as defined in Claim 1 wherein A^1 is a nitrogen heteroatom.

(3) The ^{18}F-labelled compound as defined in Claim 2 wherein A^4 is C-^{18}F.

(4) The ^{18}F-labelled compound as defined in Claim 1 wherein A^3 is a nitrogen heteroatom.

(5) The ^{18}F-labelled compound as defined in Claim 4 wherein A^4 is C-^{18}F.

(6) The ^{18}F-labelled compound as defined in Claim 1 wherein A^4 is a nitrogen heteroatom.

(7) The ^{18}F-labelled compound as defined in Claim 6 wherein A^3 is C-^{18}F.

(8) The ^{18}F-labelled compound as defined in any one of Claims 1-7 wherein R^1 is a straight or branched C_{2-4} alkyl.

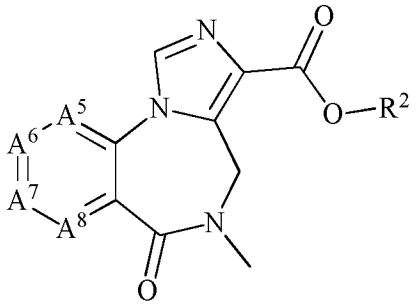
(9) The ^{18}F-labelled compound as defined in Claim 8 wherein R^1 is selected from ethyl, isopropyl and *t*-butyl.

(10) The ^{18}F-labelled compound as defined in Claim 9 wherein R^1 is ethyl.

(11) The ^{18}F-labelled compound as defined in Claim 9 wherein R^1 is *t*-butyl.

(12) A precursor compound suitable for obtaining an ^{18}F-labelled compound as defined in any one of Claims 1-11 wherein said precursor compound is of

Formula II:



II

wherein one of A^{5-8} is a nitrogen heteroatom, one of A^{5-8} is C-LG wherein LG is a leaving group, and the remaining two of A^{5-8} are CH; and,

R^2 is as defined in any one of Claims 1 and 8-11 for R^1.

(13) The precursor compound as defined in Claim 12 wherein LG is selected from halogen, nitro, tri-C_{1-3} alkyl ammonium and -I^+-Ar, wherein Ar is phenyl substituted with one or more R^* groups, wherein R^* is selected from hydrogen, nitro, cyano, halogen, C_{1-10} hydroxyalkyl, C_{2-10} carboxyalkyl, C_{1-10} alkyl, C_{2-10} alkoxyalkyl, C_{1-10} hydroxyalkyl, C_{1-10} aminoalkyl, C_{1-10} haloalkyl, C_{6-14} aryl, C_{3-12} heteroaryl, C_{3-20} alkylaryl, C_{2-10} alkenyl, and C_{2-10} alkynyl.

(14) The precursor compound as defined in Claim 13 wherein LG is halogen selected from fluoro, chloro and bromo.

(15) The precursor compound as defined in Claim 13 wherien LG is nitro.

(16) A method to obtain an ^{18}F-labelled compound as defined in any one of Claims 1-11 comprising reaction of a precursor compound as defined in any one of Claims 12-15 with a suitable source of [^{18}F]fluoride.

(17) The method as defined in Claim 16 wherein said suitable source of [^{18}F]fluoride is selected from [^{18}F]potassium fluoride and [^{18}F]caesium fluoride.

(18) The method as defined in Claim 17 wherein said suitable source of [^{18}F]fluoride is [^{18}F]potassium fluoride and wherein Kryptofix™ is used to activate the [^{18}F]fluoride ion.

(19) The method as defined in any one of Claims 16-18, which further comprises:

(i) removal of excess [^{18}F]fluoride; and/or,

(ii) removal of organic solvent; and/or,

(iii) formulation of the resultant compound together with a biocompatible carrier to obtain a radiopharmaceutical composition suitable for mammalian administration.

(20) The method as defined in any one of Claims 16-19 which is automated.

(21) A cassette suitable for carrying out the method as defined in Claim 20 wherein said cassette comprises:

(i) a vessel containing a precursor compound, wherein said precursor compound is as defined in any one of Claims 12-15; and

(ii) means for eluting the vessel with a suitable source of [^{18}F]fluoride, wherein said suitable source of [^{18}F]fluoride is as defined in the method of any one of Claims 16-18.

(22) The cassette as defined in Claim 21 which additionally comprises:

(iii) an ion-exchange cartridge for removal of excess [^{18}F]fluoride.

(23) A radiopharmaceutical composition comprising the ^{18}F-labelled compound as defined in any one of Claims 1-11 together with a biocompatible carrier in a form suitable for intravenous administration to a mammal.

(24) An *in vivo* imaging method comprising:

(i) administering to a subject the ^{18}F-labelled compound as defined in any one of Claims 1-11;

(ii) allowing said administered ^{18}F-labelled compound to bind specifically to $GABA_A$ receptors in said subject;

(iii) detecting signals derived from the positron emission decay of ^{18}F present in said specifically bound ^{18}F-labelled compound; and,

(iv) generating an image of the location and amount of said signals, wherein said signals represent the distribution of $GABA_A$ receptors in said subject.

(25) The *in vivo* imaging method as defined in Claim 24 wherein said ^{18}F-labelled compound is administered as the radiopharmaceutical composition as defined in

Claim 23.

(26) The *in vivo* imaging method as defined in either Claim 24 or Claim 25 wherein said subject is known or is suspected to have a $GABA_A$ condition.

(27) A diagnostic method comprising the *in vivo* imaging method as defined in any one of Claims 24-26 and the additional step of:

(v) attributing the distribution of $GABA_A$ receptors in said subject to a particular diagnosis.

(28) A treatment selection method comprising the *in vivo* imaging method as defined in any one of Claims 24-26.

(29) A treatment monitoring method comprising the *in vivo* imaging method as defined in any one of Claims 24-26 which is carried out before and/or during and/or after treatment of said subject with one or more drugs to combat a condition associated with abnormal expression of $GABA_A$ receptors.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/055789

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07B59/00 A61K51/04 A61K101/02 C07D487/14
ADD.

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)
C07B 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 practicable, search terms used)
EPO-Internal, CHEM ABS Data, 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	EP 0 059 388 A1 (HOFFMANN LA ROCHE [CH]) 8 September 1982 (1982-09-08) claims 1,13,14	1-29
A	GB 2 174 695 A (MERCK SHARP & DOHME) 12 November 1986 (1986-11-12) example 1, step B; example 2, step A;claim 7	1-29
Y	WO 2005/097713 A1 (GE HEALTHCARE LTD [GB]; WADSWORTH HARRY JOHN [GB]; DEVENISH TONY [GB]) 20 October 2005 (2005-10-20) claims 7,16-19	1-29

-/--

[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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 June 2012	12/06/2012

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	Schuemacher, Anne

2

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	RYZHIKOV N N ET AL: "Preparation of highly specific radioactivity [<18>F]flumazenil and its evaluation in cynomolgus monkey by positron emission tomography", NUCLEAR MEDICINE AND BIOLOGY, ELSEVIER, NY, US, vol. 32, no. 2, 1 February 2005 (2005-02-01), pages 109-116, XP027674850, ISSN: 0969-8051 [retrieved on 2005-02-01] the whole document -----	1-29
Y,P	WO 2011/042529 A1 (GE HEALTHCARE LTD [GB]; WOODCRAFT JOHN [GB]; JONES CLARE [GB]; GAETA A) 14 April 2011 (2011-04-14) the whole document -----	1-29

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/055789

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0059388 A1	08-09-1982	AU 552046 B2	22-05-1986
		AU 8066582 A	02-09-1982
		CA 1185601 A1	16-04-1985
		DE 3263829 D1	04-07-1985
		DK 63582 A	28-08-1982
		EP 0059388 A1	08-09-1982
		JP 57156486 A	27-09-1982
		US 4362732 A	07-12-1982
		ZA 8201122 A	26-01-1983
GB 2174695 A	12-11-1986	NONE	
WO 2005097713 A1	20-10-2005	CN 1938244 A	28-03-2007
		EP 1732865 A1	20-12-2006
		JP 2007532524 A	15-11-2007
		US 2007092441 A1	26-04-2007
		US 2011196149 A1	11-08-2011
		WO 2005097713 A1	20-10-2005
WO 2011042529 A1	14-04-2011	CA 2774031 A1	14-04-2011
		WO 2011042529 A1	14-04-2011