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(54) Title: INDOLE DERIVATIVES SUITABLE FOR IMAGING NEUROINFLAMMATION

In vivo imaging agent 1 in facial nucleus
of FNA rats
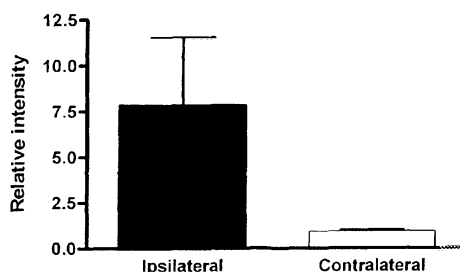
Figure 2: Relative intensity of *in vivo* imaging agent 1 binding in the facial nucleus of a rat seven days post-FNA. Data are expressed as mean ± SD of 24 individual sections from 1 animal.

(57) Abstract: The present invention concerns *in vivo* imaging and in particular *in vivo* imaging of the peripheral benzodiazepine receptor (PBR). A tetracyclic indole *in vivo* imaging agent is provided that binds with high affinity to PBR, has good uptake into the brain following administration, and which preferentially binds to tissues expressing higher levels of PBR. The present invention also provides a precursor compound useful in the synthesis of the *in vivo* imaging agent of the invention, as well as a method for synthesis of said *in vivo* imaging agent comprising use of said precursor compound, and a kit for carrying out said method. A cassette for the automated synthesis of the *in vivo* imaging agent is also provided. In addition, the invention provides a radiopharmaceutical composition comprising the *in vivo* imaging agent of the invention, as well as methods for the use of said *in vivo* imaging agent.

WO 2010/037851 A3

IMAGING NEUROINFLAMMATION

Technical Field of the Invention

The present invention concerns *in vivo* imaging and in particular *in vivo* imaging of the peripheral benzodiazepine receptor (PBR). A tetracyclic indole *in vivo* imaging agent is

5   provided that binds with high affinity to PBR, has good uptake into the brain following administration, and which preferentially binds to tissues expressing higher levels of PBR. The present invention also provides a precursor compound useful in the synthesis of the *in vivo* imaging agent of the invention, as well as a method for synthesis of said *in vivo* imaging agent comprising use of said precursor compound, and a kit for carrying

10   out said method. A cassette for the automated synthesis of the *in vivo* imaging agent is also provided. In addition, the invention provides a radiopharmaceutical composition comprising the *in vivo* imaging agent of the invention, as well as methods for the use of said *in vivo* imaging agent.

Description of Related Art

15   The peripheral benzodiazepine receptor (PBR), which is also known as translocator protein (TSPO), is known to be mainly localised in peripheral tissues and glial cells but its physiological function remains to be clearly elucidated. Subcellularly, PBR is known to localise on the outer mitochondrial membrane, indicating a potential role in the modulation of mitochondrial function and in the immune system. It has furthermore

20   been postulated that PBR is involved in cell proliferation, steroidogenesis, calcium flow and cellular respiration. PBR has been associated with a variety of conditions including acute and chronic stress, anxiety, depression, Parkinson's disease, Alzheimer's disease, brain damage, cancer (Gavish *et al* Pharm. Rev. 1999; 51 629), Huntington's disease (Meßmer and Reynolds Neurosci. Lett. 1998; 241 53-6), asthma (Pelaia *et al* Gen.

25   Pharmacol. 1997; 28(4): 495-8), rheumatoid arthritis (Bribes *et al* Eur. J. Pharmacol. 2002; 452(1): 111-22), atherosclerosis (Davies *et al* J. Nucl. Med. 2004; 45: 1898-1907) and multiple sclerosis (Banati et al 2000 Brain; 123 2321). PBR may also be associated with neuropathic pain, Tsuda *et al* having observed activated microglia in subjects with neuropathic pain (2005 TINS 28(2) pp101-7).

Ligands having high affinity for PBR are known in the art. A class of indole
compounds having affinity for PBR (IC_{50} values for most active compounds of between
0.2nM and 5.0nM) is disclosed in US 6451795. The compounds disclosed therein are
stated to be useful for the prevention or treatment of peripheral neuropathies and for the

5   treatment of central neurodegenerative diseases. Okubu *et al* (Bioorganic & Medicinal
Chemistry 2004; 12: 3569-80) describe the design, synthesis and structure of a group of
tetracyclic indole compounds having affinity for PBR (IC_{50} values as low as about
0.4nM). No particular application of the compounds is discussed in this publication by
Okubu *et al.*

10   *In vivo* imaging of PBR is also known in the art. Positron emission tomography (PET)
imaging using the PBR selective ligand, (R)-[^{11}C]PK11195 provides a generic indicator
of central nervous system (CNS) inflammation. Despite the successful use of (R)-
[^{11}C]PK11195, it has its limitations. It is known to have high protein binding, and low
specific to non-specific binding (Lockhart et al. Nucl Med Biol. 30(2):199-206). The

15   role of its radiolabelled metabolites is not known and quantification of binding requires
complex modelling. There have been efforts to provide compounds having high affinity
and selectivity for PBR to enable improved measurement of PBR in the CNS.
[^{11}C]DAA1106 and [^{18}F]FEDAA1106 are PET radioligands based on aryloxyalinine
compounds and have been studied in humans (Ikomo *et al* J. Cereb. Blood Flow Metab.

20   2007; 27: 173-84 and Fujimura *et al* J. Nuc. Med. 2006; 47: 43-50). However, the
kinetic properties of these compounds are not ideal and may limit their application to
quantitative studies. In an effort to improve further upon these radioligands, another
aryloxyaniline derivative, PBR28, has been reported by Briard *et al* (J. Med. Chem.
2008; 51: 17-30). An ^{11}C-labelled version of PBR28 was injected into monkey to

25   assess its brain kinetics using PET. [^{11}C]PBR28 showed high brain uptake, good
specific binding to PBR-expressing tissues and kinetic properties more suitable for *in
vivo* imaging. PBR-binding pyrazolopyrimidine compounds have also been evaluated
as PET radioligands for targeting PBR. James *et al* (J. Nuc. Med. 2008; 49(5): 814-22)
report that the PET radioligand [^{18}F]-DPA-714 has high affinity for PBR, and selective

30   uptake by PBR in baboon brain following intravenous administration. The kinetics of
brain uptake of [^{18}F]-DPA-714 was reported to be slower than, but similar in nature to,

[^{11}C]DAA1106 and [^{18}F]FEDAA1106. WO 2007/057705 discloses tetracyclic indole compounds labelled with an imaging moiety, which are suitable for *in vivo* imaging. The *in vivo* imaging agents exemplified in WO 2007/057705 were shown to have good affinity to PBR, with K_i values in a competition assay against [^{3}H]-PK-11195 of between 1.0nM and 0.1nM. However, the present inventors have now found that the selectivity of these compounds for PBR-expressing tissues is not ideal for *in vivo* imaging of PBR expression in the central nervous system.

There is scope to improve upon the known tetracyclic indole compounds in order to provide alternative *in vivo* imaging agents for evaluation of PBR expression in the central nervous system.

Summary of the Invention

The present invention provides *in vivo* imaging agents based on tetracyclic indole compounds. In comparison to known *in vivo* imaging agents based on tetracyclic indole compounds, the *in vivo* imaging agents of the present invention have better properties for *in vivo* imaging. The *in vivo* imaging agents of the present invention have good binding properties to the peripheral benzodiazepine receptor, as well as good brain uptake and *in vivo* kinetics following administration to a subject.

In one aspect, the present invention provides an *in vivo* imaging agent of Formula I:
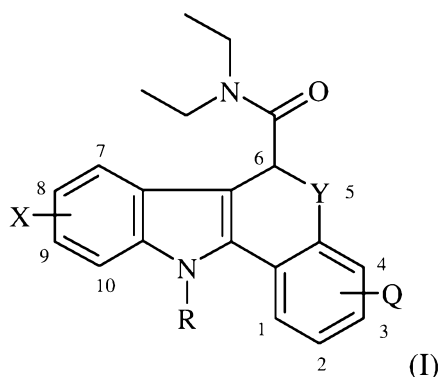
(I)

or a salt or solvate thereof wherein:

Q is hydrogen or fluorine;

X is hydrogen or C_{1-4}alkoxy

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Y is S, and,

R is C_{1-6}fluoroalkyl;

and wherein at least one atom of said *in vivo* imaging agent of Formula I is a radio isotope suitable for *in vivo* imaging which is either a gamma-emitting radioactive halogen or a positron-emitting radioactive non-metal; wherein when said radioisotope is a radio isotope of carbon, it is a carbonyl carbon; with the proviso that Q or X are not both hydrogen.

Detailed Description of the Invention

<u>*In Vivo* Imaging Agent</u>

In one aspect the present invention provides an *in vivo* imaging agent of Formula I:

(I)

or a salt or solvate thereof wherein:

Q is hydrogen or fluorine;

X is hydrogen, fluoro, bromo, iodo, hydroxy, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, or C_{1-6} alkyl amide;

5   Y is S, SO or SO_2; and,

R is hydrogen, C_{1-6} alkyl, or C_{1-6} fluoroalkyl;

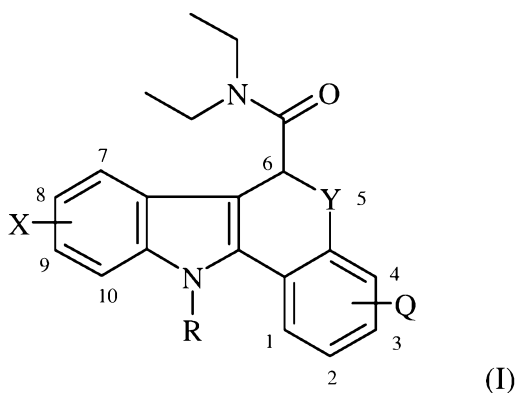and wherein at least one atom of said *in vivo* imaging agent of Formula I is a radioisotope suitable for *in vivo* imaging; wherein when said radioisotope is a radioisotope of carbon, it is a carbonyl carbon;

10   with the proviso that, when Y is S, Q or X are not both hydrogen.

The term "*in vivo* imaging" as used herein refers to those techniques that non-invasively produce images of all or part of the internal aspect of the subject of the invention. Preferred *in vivo* imaging methods for use in the present invention are single photon emission computed tomography (SPECT) and positron emission tomography (PET),
15   with PET being especially preferred. The preference for PET in the method of the invention is due to its excellent sensitivity and resolution, so that even relatively small changes in a lesion can be observed over time. 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.

20   The "*in vivo* imaging agent" of Formula I comprises a radioisotope suitable for *in vivo* imaging. This "radioisotope suitable for *in vivo* imaging" is a radioisotopic form of one of the atoms defined above for the *in vivo* imaging agent of Formula I. In order to be suitable for *in vivo* imaging as defined herein, the radioisotope is preferably a gamma- or a positron-emitter, thereby enabling detection of the *in vivo* imaging agent external to
25   the subject following administration.

Suitable salts according to the invention include (i) physiologically acceptable acid addition salts such as those derived from mineral acids, for example hydrochloric,

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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

5   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.

Suitable solvates according to the invention include those formed with ethanol, water,
10   saline, physiological buffer and glycol.

Unless otherwise specified, the term "alkyl" alone or in combination, means a straight-chain or branched-chain alkyl radical containing preferably from 1 to 6 carbon atoms, most preferably 1 to 4 carbon atoms. Examples of such radicals include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl,
15   pentyl, iso-amyl, hexyl.

Unless otherwise specified, the term "alkoxy", alone or in combination, means an alkyl ether radical wherein the term alkyl is as defined above. 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.

20   "Alkyl amide" is an alkyl group as defined above linked to an amide, wherein an amide is the group –C(=O)-NR'R" wherein R' and R" are independently hydrogen or a hydrocarbon radical.

The term "halogen" or "halo-" means a substituent selected from fluorine, chlorine, bromine or iodine. "Haloalkyl" is an alkyl group as defined above substituted with one
25   or more halogens.

The term "hydroxy" refers to the –OH radical.

In a preferred embodiment, Q is hydrogen.

X is preferably hydrogen, fluoro, bromo, iodo, hydroxy, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, or C_{1-4} alkyl amide. X is most preferably hydrogen or C_{1-4} alkoxy.

Y is preferably S or SO_2. Y is most preferably S.

R is preferably hydrogen, C_{1-4} alkyl, or C_{1-4} fluoroalkyl. R is most preferably C_{1-4}

5   fluoroalkyl.

In a preferred embodiment of Formula I:

    X is hydrogen, fluoro, bromo, iodo, hydroxy, C_{1-4} alkyl, C_{1-4} haloalkyl, C_{1-4} alkoxy, or C_{1-4} alkyl amide;

    Y is S or SO_2; and,

10   R is hydrogen, C_{1-4} alkyl, or C_{1-4} fluoroalkyl.

In a most preferred embodiment of Formula I:

    Q is hydrogen;

    X is C_{1-4} alkoxy;

    Y is S; and,

15   R is C_{1-4} fluoroalkyl.

In an alternative preferred embodiment of Formula I:

    Q is fluorine;

    X is hydrogen;

    Y is S; and,

20   R is C_{1-4} fluoroalkyl.

Preferred radioisotopes suitable for *in vivo* imaging of the present invention are gamma-emitting radioactive halogens and positron-emitting radioactive non-metals.

Examples of gamma-emitting radioactive halogens suitable for use in the present invention are ^{123}I, ^{131}I and [77]Br. A preferred gamma-emitting radioactive halogen is [123]I.

Examples of positron-emitting radioactive non-metal suitable for use in the present invention are ^{11}C, ^{13}N, ^{18}F and [124]I. A preferred positron-emitting radioactive non-metal is [18]F. [18]F is the most preferred radioisotope suitable for *in vivo* imaging of the present invention.

In a preferred embodiment, for the *in vivo* imaging agent of Formula I, X is ^{123}I, [124]I or ^{131}I, ^{18}F or C_{1-4} [^{18}F]-fluoroalkyl.

In an alternative preferred embodiment, for the *in vivo* imaging agent of Formula I, R is C_{1-4} [^{18}F]-fluoroalkyl.

In a further alternative preferred embodiment, for the *in vivo* imaging agent of Formula I the carbonyl carbon is [11]C.

Non-limiting examples of some preferred *in vivo* imaging agents of the present invention are as follows:
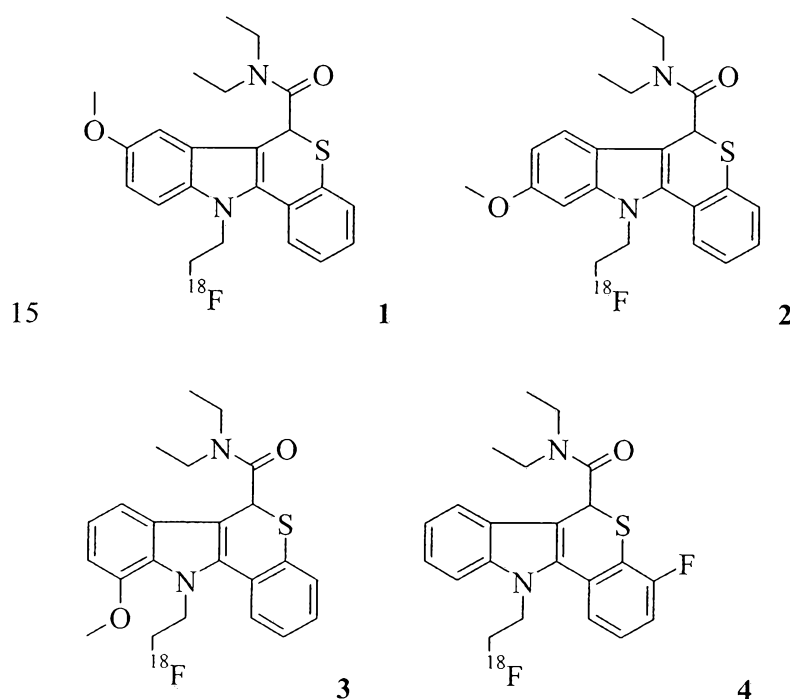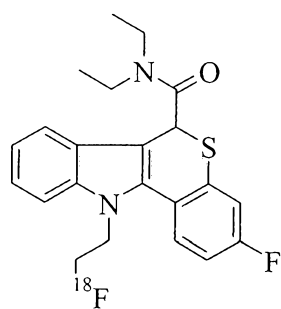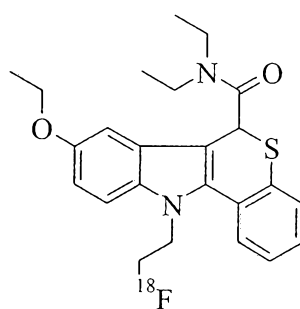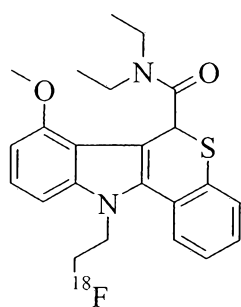
Out of the above *in vivo* imaging agents, imaging agent 2 is most preferred.

The synthetic methods used to obtain these *in vivo* imaging agents are described in the experimental section below. The potency of these non-radioactive versions of the *in vivo* imaging agents of the present invention was measured in an *in vitro* assay, as described in Example 10.

Examples 7-9 describe how to obtain the radiofluorinated *in vivo* imaging agents 1-7. The skilled person will know that when handling ^{18}F the scale and the conditions used are different for safety and practical considerations. For a review of the production of ^{18}F PET tracers, see chapters 1 and 2 of "Principles and Practice of Positron Emission Tomography" (2002 Lippincott Williams & Wilkins; Wahl and Buchanan, Eds.). The *in vivo* imaging agents were tested in an animal biodistribution model (Example 11), and their biodistribution compared to that of the prior art compound [^{18}F]FE-PBR (prepared according to Example 14 of WO 2007/057705):

[^{18}F]FE-PBR
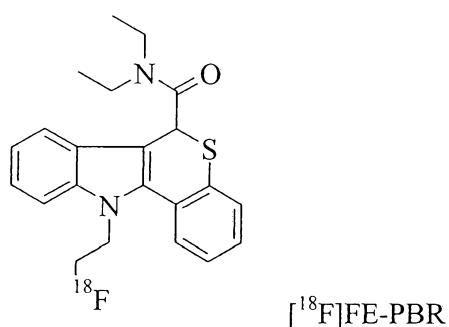
Table 1 below provides data obtained in the *in vitro* affinity assay as well as in the *in vivo* biodistribution study. Non-radioactive analogues were tested in the *in vitro* affinity assay, and radiolabelled versions were evaluated in the biodistribution assay.

In Vivo Imaging Agent	Ki	OB:Striatum
	nM	*@ 30 min*
[^{18}F]FE-PBR	0.68	1.42
1	0.37	2.07
2	0.40	3.50
3	0.93	2.00
4	0.31	2.92
5	0.32	2.26
6	0.52	2.67
7	1.09	2.42

5   **Table 1: *in vitro* affinity data and *in vivo* specific uptake data for *in vivo* imaging agents 1-7 of the present invention as compared with the prior art compound [^{18}F]FE-PBR. OB = olfactory bulb.**

The data illustrate that the potency of non-radioactive versions of *in vivo* imaging agents 1-7 compares favourably with that of the prior art compound [^{18}F]FE-PBR. In addition,

10   the data show that *in vivo* imaging agents 1-7 of the invention are retained significantly more in the OB as compared with the striatum at 30 minutes post-injection compared with [^{18}F]FE-PBR.] As it is known that PBR is highly expressed in the OB compared with other areas of the rat brain (see "Handbook of Substance Abuse" by Tarter, Ammerman and Ott; Springer 1998: 398-99) these data surprisingly demonstrate that *in*

15   *vivo* imaging agents 1-7 have improved selectivity for PBR than the previously-exemplified *in vivo* imaging agent, [^{18}F]FE-PBR.

In vivo imaging agent 1 was further analysed in an autoradiography model, as described

in Example 12 below. Significantly higher levels of radioactivity were detected in the lesioned side of the facial nucleus (see Figures 1 and 2). Average intensity in the lesioned side was 7.75 ±0.95 as compared to 3.73 ± 0.36 in the non-lesioned side. The ratio between the two sides was 8.23 ± 2.36. As the lesion has a higher expression of

5   PBR compared with normal, these data support the conclusion from the biodistribution data that *in vivo* imaging agent 1 has good selectivity for PBR.

The *in vivo* imaging agents of the present invention therefore have unexpectedly superior properties for *in vivo* imaging of PBR in comparison to known tetracyclic indole PBR-binding *in vivo* imaging agents.

10   <u>Precursor Compound</u>

In another aspect, the present invention provides a precursor compound of Formula II:
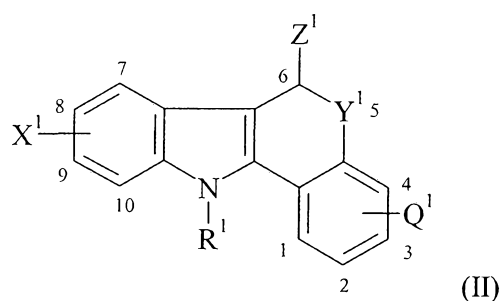
(II)

wherein one of R^1, X^1 or Z^1 comprises a chemical group that reacts with a suitable source of a radioisotope, where said radioisotope is as suitably and preferably

15   defined herein, such that the *in vivo* imaging agent as suitably and preferably defined herein is formed upon reaction of said precursor compound with said suitable source of said radioisotope;

and wherein:

when R^1 does not comprise said chemical group it is as suitably and preferably

20   defined herein for R of Formula I, and optionally further comprises a protecting group;

when X^1 does not comprise said chemical group it is as suitably and preferably

defined herein for X of Formula I, and optionally further comprises a protecting group;

when Z^1 does not comprise said chemical group it is $-C(=O)-N-(CH_2-CH_3)_2$, and optionally further comprises a protecting group;

5   Q^1 is as suitably and preferably defined herein for Q of Formula I; and,

Y^1 is as suitably and preferably defined herein for Y of Formula I, and optionally further comprises a protecting group.

A "<u>precursor compound</u>" comprises a derivative of a radiolabelled compound, designed so that chemical reaction with a convenient chemical form of the detectable label occurs

10   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. 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

15   compound.

By the term "<u>protecting group</u>" 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 product is

20   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,

25   suitable protecting groups are: methyl, ethyl or *tert*-butyl; alkoxymethyl or alkoxyethyl; benzyl; acetyl; benzoyl; trityl (Trt) or trialkylsilyl such as tetrabutyldimethylsilyl. The use of further protecting groups are described in 'Protective Groups in Organic Synthesis', Theorodora W. Greene and Peter G. M. Wuts, (Third Edition, John Wiley & Sons, 1999).

The term "a suitable source of a radioisotope" means the radioisotope in a chemical form that is reactive with a substituent of the precursor compound such that the radioisotope becomes covalently attached to the precursor compound.

For each particular radioisotope presented in the following section, one or more suitable
5   sources of the radioisotope are discussed. The person skilled in the art of *in vivo* imaging agents will be familiar with these and other sources of radioisotopes that are suitable for application in the present invention.

When the radioisotope of the *in vivo* imaging agent is ^{18}F, the radiofluorine atom may form part of a fluoroalkyl or fluoroalkoxy group, since alkyl fluorides are resistant to *in*
10   *vivo* metabolism. Alternatively, the radiofluorine atom may attach *via* a direct covalent bond to an aromatic ring such as a benzene ring.

Radiofluorination may be carried out *via* direct labelling using the reaction of ^{18}F-fluoride with a suitable chemical group in the precursor compound having a good leaving group, such as an alkyl bromide, alkyl mesylate or alkyl tosylate.

15   ^{18}F can also be introduced by O-alkylation of hydroxyl groups with ^{18}F(CH$_2$)$_3$OMs or ^{18}F(CH$_2$)$_3$Br.

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. Such a strategy is suitable for example to introduce ^{18}F at positions 1-4 or
20   7-10 of Formula I.

Alternatively, labeling with ^{18}F can be achieved by nucleophilic displacement of a leaving group from a derivative of Formula I. Suitable leaving groups include Cl, Br, I, tosylate (OTs), mesylate (OMs), and triflate (OTf). Such derivatives are precursor compounds for the preparation of *in vivo* imaging compounds of the invention.

25   Another strategy would be to have a suitable leaving group as defined above in place on an alkylamide group present on the precursor compound. 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 on the compound. Suitable protecting groups are those already mentioned previously.

When the radioisotope is ^{18}F, it is preferred that either X^1 or R^1 comprises either:

(i) an alkyl halide or an alkyl sulfonate (such as alkyl bromide, alkyl mesylate or alkyl tosylate) for neucleophilic substitution; or,

(ii) hydroxyl (for introduction of ^{18}F by O-alkylation of hydroxyl groups with e.g. $^{18}F(CH_2)_3OMs$ or $^{18}F(CH_2)_3Br$).

A generic reaction scheme to arrive at certain ^{18}F *in vivo* imaging agents of the invention is illustrated below:
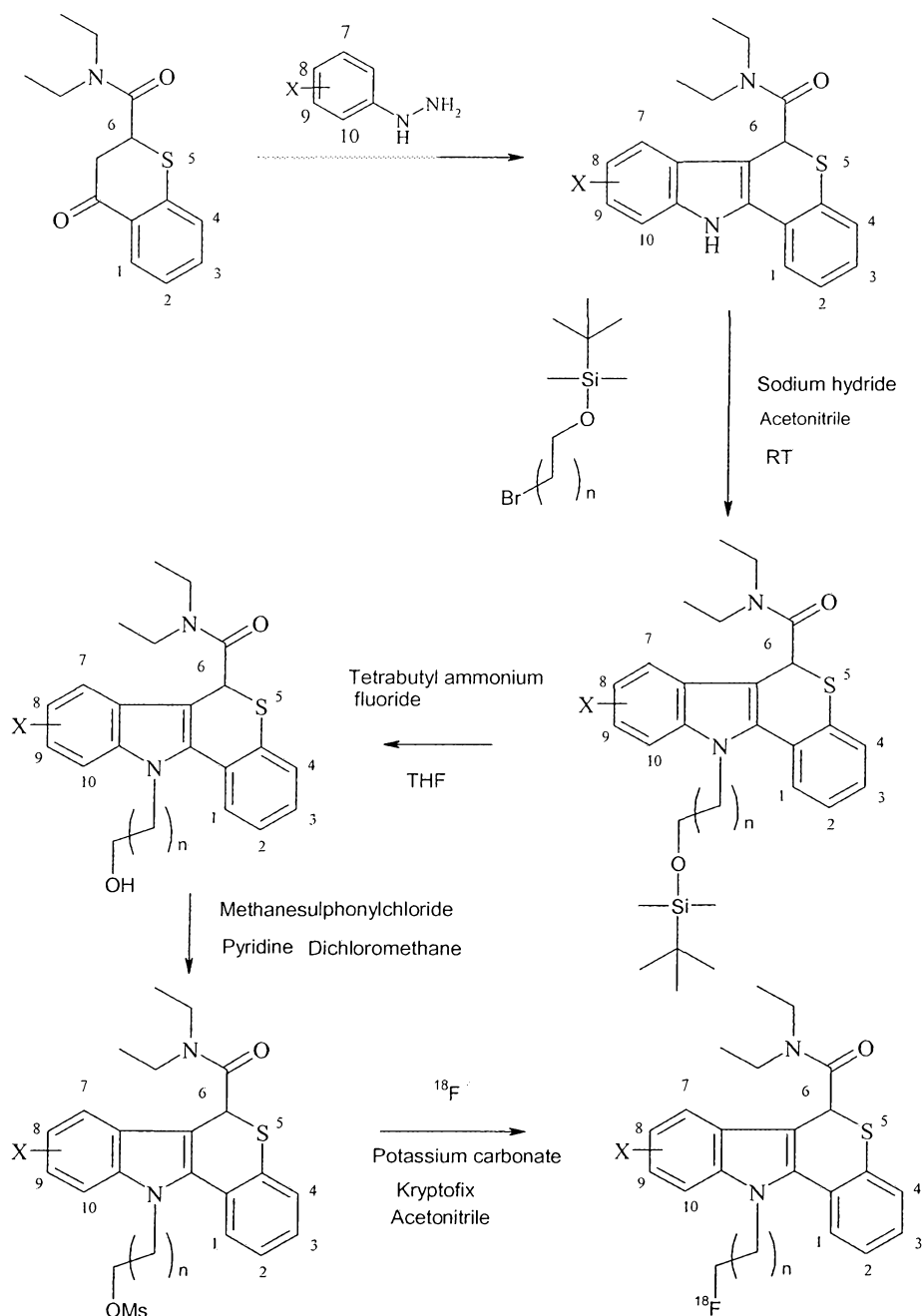
wherein X is as defined for Formula I, and n is between 0 and 5. RT stands for room temperature, and OMs stands for mesylate.

An alternative generic reaction scheme to arrive at certain [18]F *in vivo* imaging agents of the invention is illustrated below:
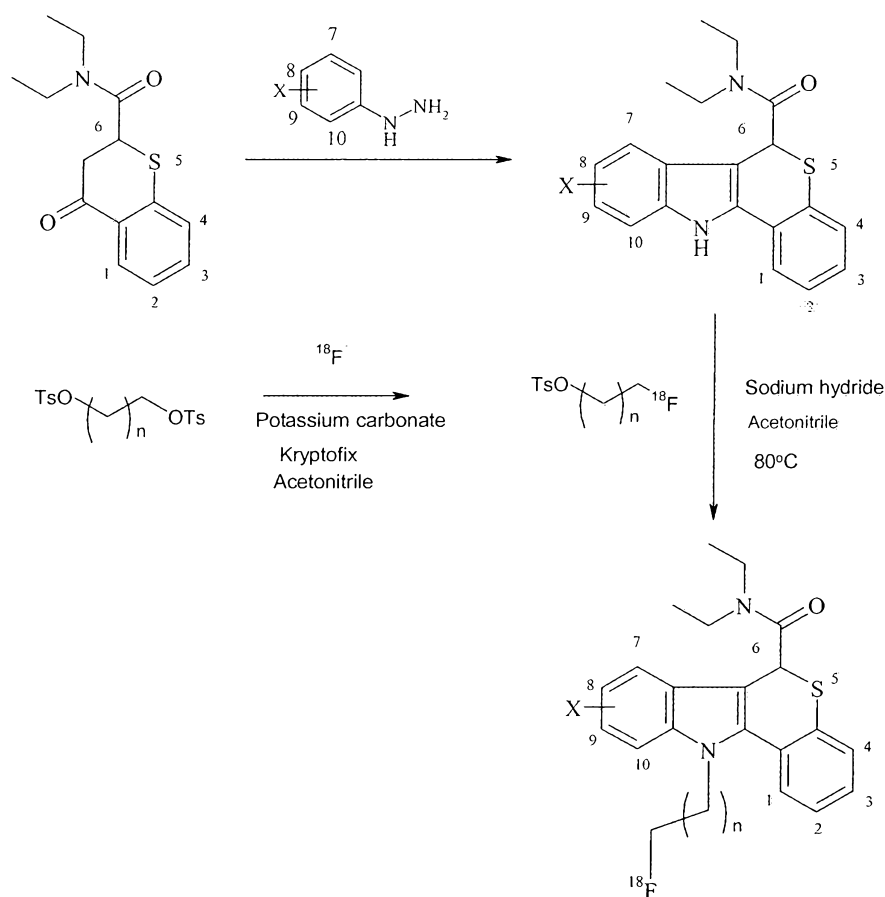
wherein X is as defined for Formula I, and n is between 0 and 5, and OTs stands for tosylate.

[11]C-labelled PET tracer compounds may be synthesised by reacting a precursor
5   compound with ^{11}C methyl iodide. As the half-life of ^{11}C is only 20.4 minutes, it is
important that the intermediate ^{11}C methyl iodide has high specific activity and,
consequently, that it is 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
10   Radiopharmaceuticals, Ed. M.J. Welch and C.S. Redvanly (2003, John Wiley and
Sons).

When the *in vivo* imaging agent of the present invention is labeled with ^{11}C, the ^{11}C is a
carbonyl carbon. This therefore means that ^{11}C can be present at the carbonyl carbon of

Formula I, or alternatively at X when X is a C_{1-6} alkyl amide.

A ^{11}C-labelled *in vivo* imaging agent of Formula I may be obtained by employing the following reaction scheme:

IIb   **Id**
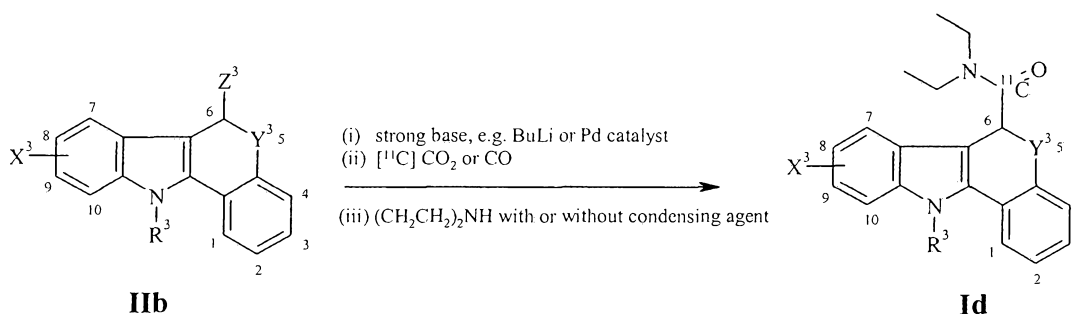
5

wherein R^3, X^3, and Y^3 of Formula IIb and Formula Id are as described for R, X, and Y of Formula I; and

Z^3 is a substrate suitable for transition metal catalysts, e.g. hydrogen, halide, boronic acid, OTf, organotin.

10   Methods for the synthesis of ^{13}N-labelled compounds are described by Clark and Aigbirhio ("Chemistry of Nitrogen-13 and Oxygen-15" in "Handbook of Radiopharmaceuticals"; 2003 Wiley: Welch and Redvanly, Eds.). For example, an *in vivo* imaging agent of Formula I may be obtained by nucleophilic substitution of a halogen in a suitable precursor compound with ^{13}N-labelled diethyl amine to obtain the

15   desired amide.

Where the imaging moiety is radioiodine, preferred precursor compounds are those which comprise a derivative which either undergoes electrophilic or nucleophilic iodination or undergoes condensation with a labelled aldehyde or ketone. Examples of the first category are:

20   (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);

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(b) aromatic rings activated towards electrophilic iodination (e.g. phenols) and

aromatic rings activated towards nucleophilic iodination (e.g. aryl iodonium salt

aryl diazonium, aryl trialkylammonium salts or nitroaryl derivatives).

For radioiodination, the precursor compound preferably comprises: an aryl iodide or

5   bromide (to permit radioiodine exchange); an activated precursor compound aryl ring

(e.g. a phenol group); an organometallic precursor compound (e.g. trialkyltin,

trialkylsilyl or organoboron compound); or an organic precursor compound such as

triazenes or a good leaving group for nucleophilic substitution such as an iodonium salt.

Precursor compounds and methods of introducing radioiodine into organic molecules

10   are described by Bolton (J. Lab. Comp. Radiopharm. 2002; 45: 485-528). Precursor

compounds and methods of introducing radioiodine into proteins are described by

Wilbur (Bioconj. Chem. 1992; 3(6): 433-470). Suitable boronate ester organoboron

compounds and their preparation are described by Kabalaka et al (Nucl. Med. Biol.,

2002; 29: 841-843 and 2003; 30: 369-373). Suitable organotrifluoroborates and their

15   preparation are described by Kabalaka et al (Nucl. Med. Biol., 2004; 31: 935-938).

Preferred precursor compounds for radioiodination comprise an organometallic

precursor compound, most preferably a trialkyltin.

Examples of aryl groups to which radioactive iodine can be attached are given below:
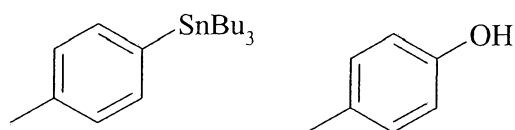
20   Both contain substituents which permit facile radioiodine substitution onto the aromatic

ring. Alternative substituents containing radioactive iodine can be synthesised by direct

iodination via radiohalogen exchange, e.g.

When the radioisotope is radioiodine, X^1 of Formula II, together with the aromatic group to

which it is attached, forms:

(i) an aromatic ring substituted with either an organometallic derivative or an organoboron compound;

(ii)   an aromatic ring activated towards electrophilic radioiodination (e.g. phenols); or,

(iii)   an aromatic ring activated towards nucleophilic radioiodination (e.g. aryl iodonium salt aryl diazonium, aryl trialkylammonium salts or nitroaryl derivatives).
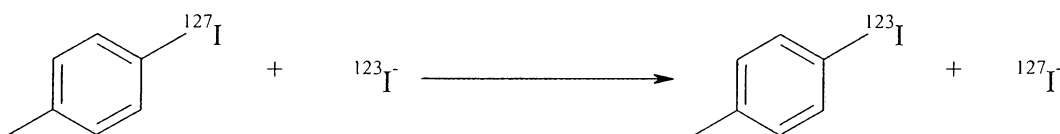
These precursor compounds are easily converted into radioiodinated *in vivo* imaging agents of the invention by radioiodine substitution.

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).

The precursor compound of the invention is ideally provided in sterile, apyrogenic form. The precursor compound can accordingly be used for the preparation of a pharmaceutical composition comprising the *in vivo* imaging agent together with a biocompatible carrier suitable for mammalian administration. The precursor compound is also suitable for inclusion as a component in a kit for the preparation of such a pharmaceutical composition.

In a preferred embodiment, the precursor compound is provided in solution and as part of a kit or of a cassette designed for use in an automated synthesis apparatus. These aspects are discussed in more detail below in relation to additional aspects of the invention.

In another preferred embodiment, the precursor compound is bound to a solid phase. The precursor compound is preferably supplied covalently attached to a solid support matrix. In this way, the desired product forms in solution, whereas starting materials

and impurities remain bound to the solid phase. As an example of such a system, precursor compounds for solid phase electrophilic fluorination with ^{18}F-fluoride are described in WO 03/002489, and precursor compounds for solid phase nucleophilic fluorination with ^{18}F-fluoride are described in WO 03/002157.

5   Method for Preparation of *In Vivo* Imaging Agent

In a further aspect, the present invention provides a method for the preparation of an *in vivo* imaging agent of the invention, said method comprising:

(i) providing a precursor compound of Formula II as defined above;

(ii) providing a suitable source of said radioisotope as defined above;

10   (iii) reacting the precursor compound of step (i) with the radioisotope of step (ii) to obtain an *in vivo* imaging agent of the invention.

In step (i), the precursor compound may be provided in solution in a kit or in a cassette suitable for use with an automated synthesis apparatus, or alternatively attached to a solid support, as described above in the description of the precursor compound. The kit

15   and cassette form additional aspects of the invention and will be discussed in more detail below.

Suitable sources of radioisotope are as described above in relation to the precursor compound of the invention.

The step of "reacting" the precursor compound with the radioisotope involves bringing

20   the two reactants together under reaction conditions suitable for formation of the desired *in vivo* imaging agent in as high a radiochemical yield (RCY) as possible. Particular synthetic routes for obtaining *in vivo* imaging agents of the present invention are presented in the experimental section below.

Kit for Preparation of *In Vivo* Imaging Agent

25   In a yet further aspect, the present invention provides a kit for the preparation of an *in vivo* imaging agent of the invention, said kit comprising a precursor compound of

Formula II as described above, so that reaction with a sterile source of a radioisotope gives the desired *in vivo* imaging agent with the minimum number of manipulations. Such considerations are particularly important where the radioisotope has a relatively short half-life, and for ease of handling and hence reduced radiation dose for the

5   radiopharmacist. The precursor compound is preferably present in the kit in lyophilized form, and the reaction medium for reconstitution of such kits is preferably a biocompatible carrier.

The "biocompatible carrier" is a fluid, especially a liquid, in which the *in vivo* imaging agent is suspended or dissolved, such that the composition is physiologically tolerable,

10   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

15   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

20   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.

In the kit of the invention, the precursor compound is preferably presented in a sealed container which permits maintenance of sterile integrity and/or radioactive safety, plus

25   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.

30   Preferred embodiments of the precursor compound when employed in the kit are as

- 20 -

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

5   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

10   contamination between runs and to ensure sterility and quality assurance.

In a preferred embodiment, the kit may comprise a cassette which can be plugged into a suitably adapted automated synthesiser, described in more detail below. Such a kit typically includes means for fluorinating with fluoride ion and may also comprise a column to remove unwanted fluoride ion. The reagents, solvents and other

15   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.

$[^{18}F]$-radiotracers for PET imaging are now often conveniently prepared on an

20   automated radiosynthesis apparatus. There are several commercially-available examples of such apparatus, including Tracerlab and Fastlab (GE Heathcare). Such apparatus commonly comprises a "cassette", often disposable, in which the radiochemistry is performed, which is fitted to the apparatus in order to perform a radiosynthesis. The cassette normally includes fluid pathways, a reaction vessel, and

25   ports for receiving reagent vials as well as any solid-phase extraction cartridges used in post-radiosynthetic clean up steps.

The present invention therefore provides in another aspect a cassette for an automated synthesis apparatus comprising the precursor compound in a sealed container as described hereinbefore. The present invention also provides a cassette for the

- 21 -

automated synthesis of an *in vivo* imaging agent as defined herein comprising:

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

(ii) means for eluting the vessel with a suitable source of a radioisotope, said radioisotope as defined herein.

5   The cassette may additionally comprise:

(iii) an ion-exchange cartridge for removal of excess radiolabel; and optionally,

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

Radiopharmaceutical Composition

10   In another further aspect, the present invention provides a "radiopharmaceutical composition", which is a composition comprising the *in vivo* imaging agent of the invention, together with a biocompatible carrier in a form suitable for mammalian administration. The biocompatible carrier is as defined above in relation to the kit of the invention.

15   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

20   acid, gentisic acid or *para*-aminobenzoic acid). Where the *in vivo* imaging agent of the invention is provided as a radiopharmaceutical composition, the method for preparation of said *in vivo* imaging agent 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

25   administration, steps to ensure that the radiopharmaceutical composition is sterile and apyrogenic also need to be taken.

In Vivo Imaging Method

In a yet further aspect, the present invention provides an _in vivo_ imaging method for determining the distribution and/or the extent of PBR expression in a subject comprising:

(i) administering to said subject an _in vivo_ imaging agent as defined herein;

5   (ii) allowing said _in vivo_ imaging agent to bind to PBR in said subject;

(iii) detecting by an _in vivo_ imaging procedure signals emitted by the radioisotope of said _in vivo_ imaging agent;

(iv) generating an image representative of the location and/or amount of said signals; and,

10   (v) determining the distribution and extent of PBR expression in said subject wherein said expression is directly correlated with said signals emitted by said _in vivo_ imaging agent.

For the _in vivo_ imaging method of the invention, the _in vivo_ imaging agent is as defined earlier in the specification.

15   "Administering" 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 also across the blood-brain barrier (BBB) and into contact with PBR expressed in said subject. The _in vivo_ imaging agent of the invention is preferably administered as the pharmaceutical

20   composition of the invention, as defined herein.

Following the administering step and preceding the detecting step, the _in vivo_ imaging agent is allowed to bind to PBR. For example, when the subject is an intact mammal, the _in vivo_ imaging agent will dynamically move through the mammal's body, coming into contact with various tissues therein. Once the _in vivo_ imaging agent comes into contact

25   with PBR, a specific interaction takes place such that clearance of the _in vivo_ imaging agent from tissue with PBR takes longer than from tissue without, or with less PBR. A certain point in time will be reached when detection of _in vivo_ imaging agent specifically bound to

PBR is enabled as a result of the ratio between *in vivo* imaging agent bound to tissue with PBR versus that bound in tissue without, or with less PBR. An ideal such ratio is around 2:1.

The "detecting" step of the method of the invention involves detection of signals emitted by the radioisotope by means of a detector sensitive to said signals. This detection step can also be understood as the acquisition of signal data. Single-photon emission tomography (SPECT) and positron-emission tomography (PET) are the most suitable *in vivo* imaging procedures for use in the method of the invention. PET is a preferred *in vivo* imaging procedures for use in the method of the invention.

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 images showing the location and/or amount of signals emitted by said radioisotope. The signals emitted directly correlate with the expression of PBR such that the "determining" step can be made by evaluating the generated image.

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 PBR in healthy subjects, or in subjects known or suspected to have a pathological condition associated with abnormal expression of PBR (a "PBR condition"). Preferably, said method relates to the *in vivo* imaging of a subject known or suspected to have a PBR condition, and therefore has utility in a method for the diagnosis of said condition. Examples of such PBR conditions where *in vivo* imaging would be of use include neuropathologies such as Parkinson's disease, multiple sclerosis, Alzheimer's disease and Huntington's disease where neuroinflammation is present. Other PBR conditions that may be usefully imaged with the compounds of the invention include neuropathic pain, arthritis, asthma, atherosclerosis, as well as malignant diseases such as colorectal cancer and breast cancer. The *in vivo* imaging agents of the invention are particularly suited to *in vivo* imaging of the central nervous system (CNS) due to their good brain uptake.

In an alternative embodiment, the *in vivo* imaging method of the invention may be carried out repeatedly during the course of a treatment regimen for said subject, said regimen comprising administration of a drug to combat a PBR condition. For example, the *in vivo* imaging method of the invention can be carried out before, during and after treatment with

5   a drug to combat a PBR condition. In this way, the effect of said treatment can be monitored over time. Preferably for this embodiment, the *in vivo* imaging procedure is PET. PET 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-

10   PET scanners now approach a spatial resolution of about 1mm, and clinical scanners about 4-5mm.

In a further aspect, the present invention provides a method for diagnosis of a PBR condition. The method of diagnosis of the invention comprises the method of *in vivo* imaging as defined above, together with the further step (vi) of attributing the distribution

15   and extent of PBR expression to a particular clinical picture, i.e. the deductive medical decision phase.

In another aspect, the present invention provides the *in vivo* imaging agent as defined herein for use in the method of diagnosis as defined herein.

In a yet further aspect, the present invention provides the *in vivo* imaging agent as defined

20   herein for use in the manufacture of a radiopharmaceutical composition as defined herein for use in the method of diagnosis as defined herein.

Brief Description of the Examples

All reagents were obtained from Sigma Aldrich.

Examples 1-6 describe the synthesis of non-radioactive versions of various *in vivo*

25   imaging agents of the invention.

Examples 7-9 describe how to obtain ^{18}F-labelled *in vivo* imaging agents of the invention.

Example 10 describes the *in vitro* potency assay used to measure PBR affinity of the imaging agents of the invention.

Example 11 describes how the animal biodistribution studies were carried out.

Example 12 describes the facial nerve axotomy animal model and its use in an
5   autoradiography study.

List of Abbreviations used in the Examples

|	DCM	dichloromethane
	DMF	dimethylformamide
	DMSO	dimethyl sulfoxide
10	EtOAc	ethyl acetate
	FNA:	facial nerve axotomy
	g	gram(s)
	h	hour(s)
	HRMS	high resolution mass spectrometry
15	K222	Kryptofix 2.2.2
	M	molarity = moles of solute/litre of solution
	MHz	mega hertz
	ml	millilitre(s)
	mmol	milimole(s)
20	N	normality = number of equivalents/1L of solution
	NMR	nuclear magnetic resonance

PBR   peripheral benzodiazepine receptor

RT   room temperature

Examples

Example 1: Preparation of (+-)-11-(2-fluoroethyl)-8-methoxy-6,11-dihydro-5-thia-11-
5   *aza-benzo[a] fluorene-6-carboxylic acid diethyl amide (non-radioactive imaging*
agent 1)

Example 1(i): (+-)-4-Oxo-thiochroman-2-carboxylic acid diethyl amide
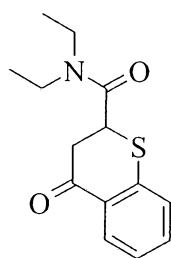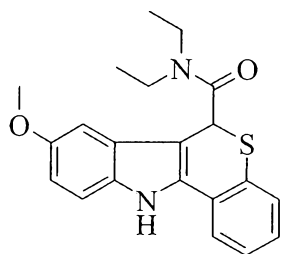

(+-)-4-Oxo-thiochroman-2-carboxylic acid (10.4 g, 50 mmol), prepared as described in
10   T. Okubo *et al* (Bioorg. Med. Chem. 2004; 12: 3569-3580), in dry DCM (100 ml) was
stirred under an atmosphere of nitrogen at room temperature with oxalyl chloride (12.6
g, 100 mmole) and one drop of DMF for 18 h. The reaction was then evaporated *in*
vacuo to a gum and then redissolved in DCM (100 ml), cooled to 0°C on an ice bath,
stirred and treated dropwise with diethylamine (8.03 g, 110 mmol) in DCM (20 ml)
15   over a period of 1 h. The reaction was allowed to warm to room temperature over 1 h
and 10% aqueous potassium carbonate solution (100 ml) was added and the reaction
mixture vigorously stirred. The DCM solution was separated. The aqueous solution
was extracted with two further batches of DCM (100 ml) and the combined extracts
were dried over magnesium sulphate. The DCM solution was concentrated *in vacuo* to
20   give a dark green oil that crystallized on standing. The crystalline solid was triturated
with diethyl ether (50 ml) and filtered to give the title compound (8.57 g, 65%) as a pale
green solid.

^{1}H NMR (300 MHz, CDCl$_3$) δ 1.06 (t, J=7.1 Hz, 3H), 1.23 (t, J=7.1 Hz, 3H), 3.0-3.5

(m, 6H), 4.25 (m, 1H), 7.15-7.21 (m, 2H), 7.32-7.39 (m, 1H), 8.10-8.14 (m, 1H).

^{13}C NMR (75 MHz, CDCl$_3$) δ 12.9, 14.8, 40.1, 40.7, 42.3, 42.5, 125.8, 127.2, 128.7, 130.8, 133.4, 137.9, 167.9, 193.1

5   *Example 1(ii): (+-)-8-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a] fluorene-6-carboxylic acid diethyl amide*


To a solution of (+-)-4-Oxo-thiochroman-2-carboxylic acid diethyl amide (1.32 g, 5.0 mmol; Example 1(i)) and 4-methoxyphenyl hydrazine hydrochloride (0.87 g, 5.0 mmol) in ethanol (10 ml) was added concentrated sulphuric acid (0.73 ml, 1.35 g, 13.8 mmol)

10   under nitrogen. The reaction mixture was heated under reflux for 24 h. After cooling, the reaction mixture was filtered, the solid washed with ethanol, dried *in vacuo* (45°C) to give the title compound (1.05 g, 57%) as a pale yellow solid.

^{1}H NMR (300 MHz, DMSO-d$_6$) δ 0.97 (t, J=6.8 Hz, 3H), 1.28 (t, J=6.8 Hz, 3H), 3.25 (m, 2H), 3.60 (m, 2H), 3.74 (s, 3H), 5.59 (s, 1H), 6.80 (m, 2H), 7.10-7.35 (m,

15   4H), 7.75 (d, J=7.3 Hz, 1H), 11.52 (s, 1H, NH).

^{13}C NMR (75 MHz, DMSO-d$_6$) δ 10.5, 12.7, 32.7, 37.9, 39.5, 53.0, 97.6, 103.3, 109.87, 109.92, 120.3, 123.5, 123.8, 124.3, 124.7, 124.9, 127.8, 129.4, 131.8, 151.3, 166.2

m/z (ES$^+$) 367.1 (M$^+$H).

Example 1(iii): (+-)-11-(2-fluoroethyl)-8-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]
fluorene-6-carboxylic acid diethyl amide
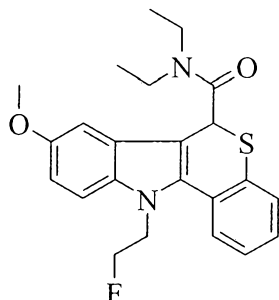
To a solution of (+-)-8-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a] fluorene-6-

5 carboxylic acid diethyl amide (150 mg, 0.41 mmol; Example 1(ii)) in anhydrous DMF

(4 ml) was added 2-fluoroethyl tosylate (166 mg, 0.82 mmol)), prepared as described in

L. Cronin _et al_ (J. Org. Chem. 2004; 69: 5934-5946) followed by sodium hydride 60%

dispersion in mineral oil (34 mg, 0.82 mmol) under nitrogen. The reaction mixture was

heated at 80°C for 1 h. After cooling, the solvents were removed _in vacuo_, the residue

10 quenched with water (30 ml), extracted with DCM (2 x 30 ml), dried (MgSO$_4$) and

solvents removed _in vacuo_. The residue was purified by column chromatography on

silica, eluting with 5-10% EtOAc/CH$_2$Cl$_2$. The crude solid was quenched with

ether/pet.spirit, filtered, dried _in vacuo_ (45°C) to give the title compound (77 mg, 46%)

as a pale brown solid.

15 ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.12 (t, J=7.0 Hz, 3H), 1.36 (t, J=7.0 Hz, 3H), 3.25-

3.70 (m, 4H), 3.83 (s, 3H), 4.45-4.70 (m, 2H), 4.80 (t, J=5.2 Hz, 1H), 4.96 (t, J=5.2

Hz, 1H), 5.09 (s, 1H), 6.84-6.93 (m, 2H), 7.13-7.32 (m, 3H), 7.46 (m, 1H), 7.58 (d,

J=8.0 Hz, 1H).

^{13}C NMR (75 MHz, CDCl$_3$) δ 12.9, 14.9, 37.3, 41.1, 42.5, 45.5, 45.8, 55.9, 81.2,

20 83.5, 100.4, 110.1, 111.09, 111.12, 112.8, 124.31, 124.35, 125.2, 126.5, 127.1,

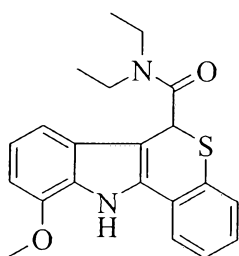127.6, 128.8, 132.2, 134.4, 137.0, 154.8, 168.0

^{19}F NMR (282 MHz, CDCl$_3$) δ -219.4, -219.5, -219.6, -219.65, -219.73, -219.8,

-219.9

m/z (ES$^+$) 413.1 (M$^+$H).

Example 2: Preparation of (+-)-11-(2-fluoroethyl)-10-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a] fluorene-6-carboxylic acid diethyl amide (non-radioactive imaging agent 3)
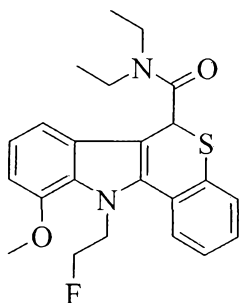
Example 2(i): (+-)-10-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a] fluorene-6-carboxylic acid diethyl amide

This compound was prepared as described for Example 1(ii) except that 2-methoxyphenyl hydrazine hydrochloride was used instead of 4-methoxyphenyl hydrazine hydrochloride. The compound was obtained in 40% yield.

m/z (ES$^+$) 367.0 (M$^+$H).

Example 2(ii): (+-)-11-(2-fluoroethyl)-10-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a] fluorene-6-carboxylic acid diethyl amide

This compound was prepared as described for Example 1(iii) except that (+-)-10-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a] fluorene-6-carboxylic acid diethyl amide

(Example 2(i)) was used instead of (+-)-8-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a] fluorene-6-carboxylic acid diethyl amide. After recrystallisation (ether), was obtained in 10% yield as a white solid.
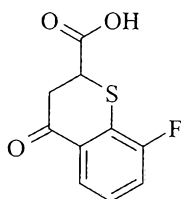
5   ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.09 (t, J=7.0 Hz, 3H), 1.35 (t, J=7.0 Hz, 3H), 3.25-3.67 (m, 4H), 3.95 (s, 3H), 4.70-4.96 (m, 4H), 5.04 (s, 1H), 6.67 (m, 1H), 7.04 (m, 2H), 7.16 (m, 1H), 7.29 (m, 1H), 7.45 (m, 1H), 7.77 (m, 1H).

m/z (ES$^+$) 413.1 (M$^+$H).

Example 3: Preparation of (+-)-4-fluoro-11-(2-fluoroethyl)-6,11-dihydro-5-thia-11-aza-benzo[a] fluorene-6-carboxylic acid diethyl amide (non-radioactive imaging

10   ***agent 4)***

*Example 3(i): (+-)-8-Fluoro-4-oxo-thiochrom৹৹৹-2-carboxlic acid*

In a round bottom flask 2-fluorothiophenol (5.0 g, 39.0 mmol, 4.16 mL) and furan-2,5-dione (3.82 g, 39.0 mmol) in toluene (12 mL) were stirred at 50°C for 40 minutes.

15   Triethylamine (100 μl) in toluene (5 mL) was then added over 10 minutes ensuring the reaction temperature did not increase over 60°C. The reaction was then heated at 70°C for 20 minutes. The reaction was then concentrated under high vacuum to obtain the crude product as an oil. This material was dissolved in DCM (75 mL), cooled on an ice bath and treated with aluminium trichloride (7.78 g, 58.5 mmol) in small portions so as

20   to keep the temperature below 10°C. The reaction was warmed to RT and there was a vigorous evolution of hydrogen chloride gas and the reaction became very viscous and turned red. After stirring at RT for 1.5 hours the reaction mixture was then diluted with DCM (50 mL) to make it less viscous and slowly poured into vigorously stirred concentrated hydrochloric acid (30 mL) and ice (30 g) in a 2L conical flask. The

25   reaction was vigorously stirred and diluted with a further portion of DCM (500 mL) and

isopropyl alcohol (50 mL) to dissolve any solid that had crystallized out. The DCM

layer was separated, dried over magnesium sulfate and concentrated in vacuum to give a

brown solid. The crude solid was purified by triturated with diethyl ether and a cream

solid was collected by filtration to give 2.5 g (28%) of 8-Fluoro-4-oxo-thiochromana-2-

5   carboxlic acid. ^{1}H NMR (300 MHz; DMSO-d₃): δ 3.04-3.20 (2H, m, 3-H), 4.51 (1H,

dd, J = 4 and 6 Hz, 2-H), 7.26-7.34 (1H, *m*, 6-H), 7.45- 7.52 (1H, *m*, 7-H), 7.82 (1H,

dd, J = 1 and 8 Hz, 5H). ^{13}C NMR (75 MHz; DMSO-d₃): δ 40.5, 40.7, 119.8, 120.1,

123.88, 123.92, 126.0, 126.1, 131.9, 156.1, 159.2, 171.5, 191.2, 191.3.

Example 3(ii): (+-)-8-Fluoro-4-oxo-thiochroman-2-carboxylic acid diethylamide
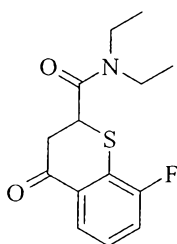
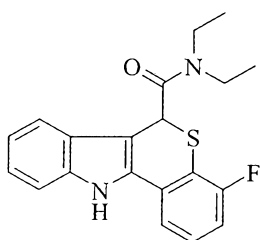
10

8-Fluoro-4-oxo-thiochromana-2-carboxlic acid (2.5 g, 11.1 mmol) in dry DCM (50ml)

was stirred under an atmosphere of nitrogen at room temperature with oxalyl chloride

(2.81 g, 22.1 mmo, 1.93 mL) and one drop of DMF to catalyse the reaction for 18h.

The acid was initially insoluble but dissolved as it reacted to give a orange clear

15   solution after 2 hours and then turned black after 24h. The reaction was then

evaporated in vacuum to a gum to remove excess oxalyl chloride and ^{1}H and ^{13}C NMR

run in CDCl₃ to confirm complete reaction. The reaction was then redissolved in DCM

(50ml) cooled to 0°C on an ice bath stirred and treated dropwise with diethylamine (1.66

g, 22.7 mmol, 2.05 mL) in DCM (20ml) over a period of 1h. The reaction was allowed

20   to warm to room temperature over a period of 1h. The reaction was then quenched by

the addition of 5% potassium carbonate solution (100ml) and the reaction mixture

stirred vigorously. The DCM solution was separated and dried over magnesium

sulphate. Two further batches of DCM (100ml) were shaken with the aqueous solution,

and then separated and dried over magnesium sulphate. The combined DCM solutions

25   were concentrated in vacuum to give a brown solid. The crude solid was purified by hot

recrystallisation from ethyl acetate and petrol to afford 1.73 g (56%) of 8-Fluoro-4-oxo-

thiochroman-2-carboxylic acid diethylamide as yellow crystals. ^{1}H NMR (300 MHz; CDCl$_3$): δ 1.07 (3H, *t, J = 7 Hz*, N(CH$_2$C$\underline{H}$$_3$)$_2$), 1.26 (3H, *t, J = 7 Hz*, N(CH$_2$C$\underline{H}$$_3$)$_2$), 3.02-3.55 (6H, *m*, 2-H and N(C$\underline{H}$$_2CH_3$)$_2$), 4.24-4.27 (1H, *m*, 2-H), 7.15-7.19 (2H, *m*, 6-H and 7-H), 7.93-7.97 (1H, *m*, 5-H).

5   LC-MS: *m/z* calcd for C$_{14}$H$_{16}$FNO$_2$S 281.1; found, 282.0 (M+H)+

Example 3(iii): (+-)-4-Fluoro-6,11-dihydro-5-thia-11azabenzo[a]fluorene-6-carboxylic acid diethylamide

8-Fluoro-4-oxo-thiochromana-2-carboxylic acid diethylamide (1.7 g, 6.0 mmol) and
10   phenyl hydrazine 0.65 g, 6.0 mmol, 0.6 mL) in ethanol (10 mL) and sulphuric acid (conc., 0.8 mL) were stirred at reflux for overnight. After cooling the reaction was filtered and the white solid was collected to afford 1.4 g (80%) of crude material (90% pure). The crude solid (500 mg) was purified by hot re-crystallisation from ethanol to afford 277 mg (13%) of 4-Fluoro-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-
15   carboxylic acid diethylamide as white crystals. The structure was confirmed by ^{1}H NMR (300 MHz; DMSO-d$_6$): δ 0.96 (3H, *t, J = 7 Hz*, N(CH$_2$C$\underline{H}$$_3$)$_2$), 1.30 (3H, *t, J = 9 Hz*, N(CH$_2$C$\underline{H}$$_3$)$_2$), 3.19-3.25 (2H, *m*, N(C$\underline{H}$$_2CH_3$)$_2$), 3.56-3.66 (2H, *m*, N(C$\underline{H}$$_2CH_3$)$_2$), 5.76 (1H, *s*, 6-H), 7.02-7.45 (6H, *m*, ArH), 7.65 (1H, *dd, J = 1 and 6 Hz*, ArH), 11.8 (1H, *s*, N$\underline{H}$).

20   LC-MS: *m/z* calcd for C$_{20}$H$_{19}$FN$_2$OS 354.2; found, 355.0(M+H)+

Example 3(iv): (+-)-4-fluoro-11-(2-fluoroethy) -6,11-dihydro-5-thia-11-aza-benzo[a]
fluorene-6-carboxylic acid diethyl amide
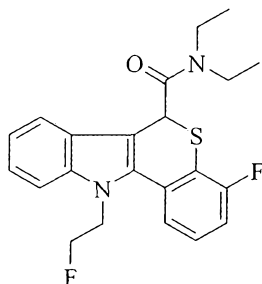

(+-)-4-Fluoro-6,11-dihydro-5-thia-11azabenzo[a]fluorene-6-carboxylic acid

5   diethylamide (0.10 g, 0.28 mmol) was dissolved in dry DMF (6 mL) at room

temperature under nitrogen. Fluoroethyl tosylate (0.12 g, 0.12 mmol) was added and

then NaH (0.02 g, 0.56 mmol, 60% in oil). The reaction was heated to 80°C for 1 hour.

The solvent was removed under reduced pressure and the residue was dissolved in

DCM and washed with water. The organics were dried over $MgSO_4$, filtered and

10   evaporated to dryness. The crude material was crystallized from methanol to afford

34.4 mg (30%) of 4-Fluoro-11-(2-fluoro-ethyl)-6,11-dihydro-5-thia-11-aza-

benzo[a]fluorene-6-carboxylic acid diethylamide as a white solid. ^{1}H NMR (300 MHz,

DMSO-d_6) δ 0.94 (3H, *t, J = 7 Hz*, N(CH$_2$CH$_3$)$_2$), 1.29 (3H, *t, J = 7 Hz*, N(CH$_2$CH$_3$)$_2$),

3.14-3.26 (2H, *m*, N(CH$_2$CH$_3$)$_2$), 3.55-3.65 (2H, *m*, N(CH$_2$CH$_3$)$_2$), 4.65-4.95 (4H, *m*,

15   NCH$_2$CH$_2$F), 5.62 (1H, *s*, 6-H), 7.12-7.37 (4H, *m*, ArH), 7.48 (1H, *d, J = 9 Hz*, ArH),

7.61-7.68 (2H, *m*, ArH).

LC-MS: *m/z* calcd for C$_{22}$H$_{22}$F$_2$N$_2$OS 401.1; found, 401.1 (M+H)+.

Example 4: Preparation of (+-)3-fluoro-11-(2-fluoroethyl)-6,11-dihydro-5-thia-11-aza-benzo[a] fluorene-6-carboxylic acid diethyl amide (non-radioactive imaging agent 5)

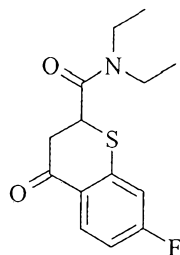*Example 4(i): (+-)-7-Fluoro-4-oxo-thiochromana-2-carboxlic acid*
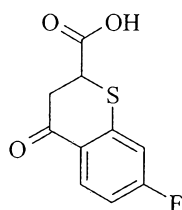
5

In a round bottom flask 3-Fluorothiophenol (10.0 g, 71.3 mmol, 8.85 mL) and furan-2,5-dione dione (7.0 g, 71.3 mmol) in toluene (12 mL) were stirred at 50°C for 40 minutes. Triethylamine (26 µl) in toluene (1 mL) was then added over 10 minutes insuring the reaction temperature did not increase over 60°C. The reaction was then

10 heated at 70°C for 20 minutes. The reaction was then concentrated under high vacuum to obtain the crude product as an oil. This material was dissolved in DCM (75 mL), cooled on an ice bath and treated with aluminium trichloride (7.78 g, 58.5 mmol) in small portions so as to keep the temperature below 10°C. The reaction was warmed to RT and there was a vigorous evolution of hydrogen chloride gas and the reaction

15 became very viscous and turned red. After stirring at room temperature for 1.5 hours the reaction mixture was then diluted with DCM (50 mL) to make it less viscous and slowly poured into vigorously stirred concentrated hydrochloric acid (30 mL) and ice (30 g) in a 2L conical flask. The reaction was vigorously stirred and diluted with a further portion of DCM (500 mL) and isopropyl alcohol (50 mL) to dissolve any solid

20 that had crystallized out. The DCM layer was separated, dried over magnesium sulfate and concentrated in vacuum to give a brown solid. The solid was triturated with diethyl ether and then filtered to give 4.2g (48%) of 7-Fluoro-4-oxo-thiochroman-2-carboxylic acid as a cream solid. ^{1}H NMR (300 MHz; DMSO-d$_3$): δ 3.00-3.16 (2H, *m*, 3-H), 4.44 (1H, *dd, J = 5 and 10 Hz*, 2-H), 7.08 (1H, *td, J$_l$ = 3 and 9 Hz*, 6-H), 7.30 (1H, *dd, J = 5*

25 *and 10 Hz*, ArH), 8.01 (1H, *dd, J$_l$ = 5 and 10 Hz*, ArH). ^{13}C NMR (75 MHz; DMSO-d$_3$): δ 38.0, 39.6, 111.1, 111.3, 111.5, 111.8, 125.0, 125.1, 129.0, 129.2, 139.6, 139.7,

160.9, 164.3, 169.5, 188.9.

Example 4(ii): (+-)-7-Fluoro-4-oxo-thiochromana-2-carboxlic acid diethylamide

7-Fluoro-4-oxo-thiochromana-2-carboxlic acid (4 g, 17.7 mmol) in dry DCM (50ml)

5   was stirred under an atmosphere of nitrogen at room temperature with oxalyl chloride

(4.49 g, 35.4 mmol, 3.1 mL) and one drop of DMF to catalyse the reaction for 18h. The

acid was initially insoluble but dissolved as it reacted to give a orange clear solution

after 2 hours and then turned black after 18h. The reaction was then evaporated in

vacuum to a gum to remove excess oxalyl chloride and ^{1}H and ^{13}C NMR run in CDCl$_3$

10   to confirm complete reaction. The reaction was then redissolved in DCM (50ml) cooled

to 0°C on an ice bath stirred and treated dropwise with diethylamine in DCM (10ml)

over a period of 1h. The reaction was allowed to warm to room temperature over a

period of 1h. The reaction was then quenched by the addition of 5% potassium

carbonate solution (50ml) and the reaction mixture stirred vigorously. The DCM

15   solution was separated and dried over magnesium sulphate. Two further batches of

DCM (50ml) were shaken with the aqueous solution, and then separated and dried over

magnesium sulphate. The combined DCM solutions were concentrated in vacuum to

give a brown solid, which crystallised on standing to afford 5.03 g (quant) of 7-fluoro-4-

oxo-thiochroman-2-carboxylic acid diethylamide. The structure was confirmed by ^{1}H

20   NMR (300 MHz; CDCl$_3$): δ 1.07 (3H, *t, J = 7 Hz*, N(CH$_2$CH$_3$)$_2$), 1.24 (3H, *t, J = 7 Hz*,

N(CH$_2$CH$_3$)$_2$), 2.99-3.50 (6H, *m*, 2-H and N(CH$_2$CH$_3$)$_2$), 4.24-4.27 (1H, *m*, 2-H), 6.83-

6.94 (2H, *m*, 6-H and 8-H), 8.15 (1H, *dd, J = 6 and 9 Hz*, 5-H).

LC-MS: *m/z* calcd for C$_{14}$H$_{16}$FNO$_2$S 281.1; found, 282.0 (M+H)+

Example 4(iii): (+-)-3-Fluoro-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-
carboxylic acid diethylamide
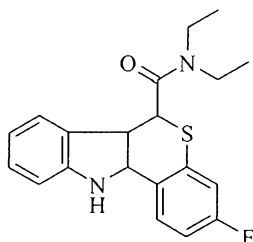
7-Fluoro-4-oxo-thiochromana-2-carboxylic acid diethylamide (2.5 g, 8.9 mmol) and
5   phenyl hydrazine 0.96 g, 8.9 mmol, 0.9 mL) in ethanol (10 mL) and sulphuric acid
(conc., 1.2 mL) were stirred at reflux for overnight. The crude solid was purified by hot
re-crystallisation from ethanol to afford 1.49 g (47%) of 3-Fluoro-6,11-dihydro-5-thia-
11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide as white crystals. ^{1}H NMR
(300 MHz; DMSO-d$_6$): δ 0.96 (3H, *t, J = 6 Hz*, N(CH$_2$CH$_3$)$_2$), 1.29 (3H, *t, J = 6 Hz*,
10   N(CH$_2$CH$_3$)$_2$), 3.19-3.25 (2H, *m*, N(CH$_2$CH$_3$)$_2$), 3.55–3.61 (2H, *m*, N(CH$_2$CH$_3$)$_2$), 5.66
(1H, *s*, 6-H), 7.03 (1H, *td, J = 1 and 8 Hz*, ArH), 7.09-7.18 (2H, *m*, ArH), 7.25 (1H, *dd,
J = 3 and 9 Hz*, ArH), 7.35 (1H, *d, J = 8 Hz*, ArH), 7.41 (1H, *d, J = 8 Hz*, ArH), 7.81
(1H, *dd, J = 6 and 9* Hz, ArH), 11.68(1H, *s*, NH).

LC-MS: *m/z* calcd for C$_{20}$H$_{19}$FN$_2$OS 352.1; found, 353.2 (M+H)+.

15   *Example 4(iv): (+-) 3-Fluoro-11-(2-fluoro-ethyl)-6,11-dihydro-5-thia-11-aza-*
benzo[a]fluorene-6-carboxylic acid diethylamide
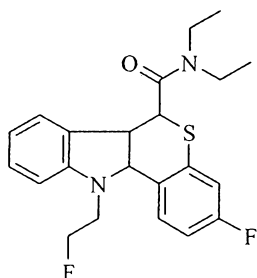
3-Fluoro-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide
(0.20 g, 0.56 mmol) was dissolved in dry DMF (6 mL) at room temperature under

nitrogen. Fluoroethyl tosylate (0.25 g, 1.13 mmol) was added and then NaH (0.05 g, 1.13 mmol, 60% in oil). The reaction was heated to 80°C for 1 hour. The solvent was removed under reduced pressure and the residue was dissolved in DCM and washed with water. The organics were dried over MgSO$_4$, filtered and evaporated to dryness. The crude material was purified by semi preparative HPLC eluting with water (A) and acetonitrile (B) (Gemini 5u, C18, 110A, 150 x 21mm, 5-95% B over 20 min, 21 mL/min) to afford 79.9 mg (35%) of 3-Fluoro-11-(2-fluoro-ethyl)-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide as a white solid. ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 0.95 (3H, *t*, *J* = *9 Hz*, N(CH$_2$CH$_3$)$_2$), 1.88 (3H, *t*, *J* = *9 Hz*, N(CH$_2$CH$_3$)$_2$), 3.14-3.26 (2H, m, N(CH$_2$CH$_3$)$_2$), 3.51-3.67 (2H, *m*, N(CH$_2$CH$_3$)$_2$), 4.58-4.97 (4H, *m*, NCH$_2$CH$_2$F), 5.53 (1H, *s*, 6-H), 7.12-7.27 (3H, *m*, ArH), 7.38-4.47 (2H, *m*, ArH), 7.61 (1H, *d*, *J* = *9* Hz, ArH), 7.80-7.86 (1H, *m*, ArH).

LC-MS: *m/z* calcd for C$_{22}$H$_{22}$F$_2$N$_2$OS 401.1; found, 401.1 (M+H)+.

Example 5: Preparation of (+-)8-ethoxy-11-(2-fluoroethyl)-6,11-dihydro-5-thia-11-aza-benzo[a] fluorene-6-carboxylic acid diethyl amide (non-radioactive imaging agent 6)
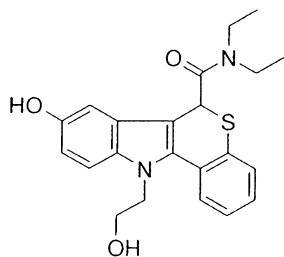
Example 5(i): (+-) 11-[2-(tertbutyl-dimethyl-silanyloxy)] ethyl]-8-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide
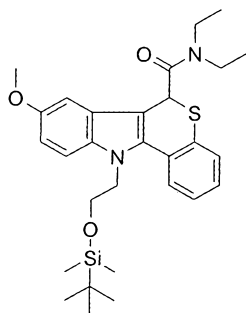
To a solution of (+-)-8-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a] fluorene-6-carboxylic acid diethyl amide Example 1(ii) (2.0 g, 5.40 mmol) in anhydrous DMF (20 ml) was added sodium hydride 60% dispersion in mineral oil (240 mg, 6.0 mmol) and the mixture stirred at room temperature for 5 min under nitrogen. 2-(bromoethoxy)-tert-
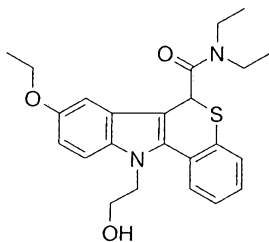
butyl-dimethylsilane (2.6 g, 10.8 mmol) was added and the mixture stirred for 4h. The

solvents were removed *in vacuo*, the residue quenched with water (30 ml), extracted

with DCM (2 x 30 ml), dried (MgSO₄) and solvents removed *in vacuo*. The residue

was purified by column chromatography on silica, eluting with 3% EtOAc/CH₂Cl₂ to

5   give the title compound (2.0g, 70 %) as a yellow solid.

Example 5(ii): (+-) 11-[2-hydroxyethyl]-8-hydroxy-6,11-dihydro-5-thia-11-aza-
benzo[a]fluorene-6-carboxylic acid diethylamide

To a solution of (+-) 11-[2-(tertbutyl-dimethyl-silanyloxy)]ethyl]-8-methoxy-6,11-

10   dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide (1.0 g, 1.91

mmol) in dry DCM (60 ml) at -78°C was added boron tribromide (11.5 ml, 1M in DCM,

11.5 mmol). The solution was allowed to rise to RT and stirred for 24 h. The solvents

were removed *in vacuo*, quenched with methanol (40 ml), and 1NHCl (10 ml) added,

refluxed for 1h. The solvents were removed *in vacuo*, the mixture was dissolved

15   methanol (5 ml), quenched with water (100 ml), filtered, dried *in vacuo* (45°C) to give
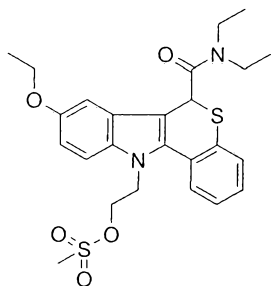
the title compound (0.77 g, 100%) as a light brown powder.

Example 5(iii): (+-) 11-[2-hydroxyethyl]-8-ethoxy-6,11-dihydro-5-thia-11-aza-
benzo[a]fluorene-6-carboxylic acid diethylamide

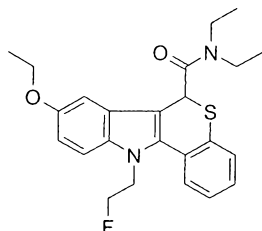20   To a solution of (+-) 11-[2-hydroxyethyl]-8-hydroxy-6,11-dihydro-5-thia-11-aza-

benzo[a]fluorene-6-carboxylic acid diethylamide (400 mg, 1.01 mmol) in anhydrous

DMF (4 ml) at 0°C was added sodium hydride 60% dispersion in mineral oil (40 mg,

1.01 mmol). The mixture was stirred at 0°C for 10 min under nitrogen. Ethyl bromide

(218 mg, 2.0 mmol, 150ul) was added and the mixture stirred for 24h. The solvents

5   were removed *in vacuo*, the residue quenched with water (30 ml), extracted with DCM

(2 x 30 ml), dried (MgSO₄) and solvents removed *in vacuo*. The residue was purified

by column chromatography on silica, eluting with 40-60% EtOAc/CH₂Cl₂ to give the

title compound (340 mg, 79 %) as a white solid.

Example 5(iv): (+-) 11-[2-methanesulphoxyethyl]-8-ethoxy-6,11-dihydro-5-thia-11-aza-

10   *benzo[a]fluorene-6-carboxylic acid diethylamide*

To a suspension of (+-) 11-[2-hydroxyethyl]-8-ethoxy-6,11-dihydro-5-thia-11-aza-

benzo[a]fluorene-6-carboxylic acid diethylamide (0.34 g, 0.80 mmol) in anhydrous

DCM (15 ml) was added pyridine (0.63 g, 8.0 mmol, 0.65 ml). The reaction was cooled

15   to 0°C and methane sulfonyl chloride (0.37 g, 3.2 mmol, 0.25 ml) was added. The

reaction mixture was stirred at RT for 3h. The mixture was washed with 0.5M HCl

(2x20 ml), then water 2x20 ml), dried (MgSO₄) and the solvent removed under reduced

pressure. The residue was purified by column chromatography on silica, eluting with

20% EtOAc/CH₂Cl₂ The residue was quenched with ether/pet. spirit, filtered, dried *in*

20   *vacuo* (45°C) to give the title compound (0.38 g, 95 %) as a pale yellow solid.
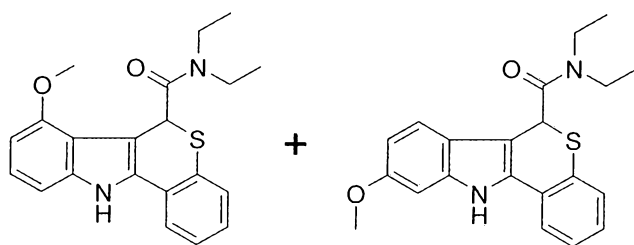
Example 5(v): (+-) 11-[2-fluoroethyl]-8-ethoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide

To a solution of (+-) 11-[2-methanesulphoxyethyl]-8-ethoxy-6,11-dihydro-5-thia-11-

5   aza-benzo[a]fluorene-6-carboxylic acid diethylamide (100 mg, 0.20 mmol) in

anhydrous acetonitrile (5 ml) under nitrogen was added TBAF 1.0 M in THF (0.4 ml,

0.4 mmol). The mixture was heated to 80°C for 2h. The solvents were removed *in*

vacuo and the residue purified by column chromatography on silica eluting with 5-10%

EtOAc/CH$_2$Cl$_2$ to give the title compound (26 mg, 31 %) as a yellow solid.

10   ***Example 6: Preparation of (+-)7-methoxy-11-(2-fluoroethyl)-6,11-dihydro-5-thia-11-***

aza-benzo[a] fluorene-6-carboxylic acid diethyl amide (non-radioactive imaging

agent 2) and (+-)9-methoxy-11-(2-fluoroethyl)-6,11-dihydro-5-thia-11-aza-benzo[a]

fluorene-6-carboxylic acid diethyl amide (non-radioactive imaging agent 7)
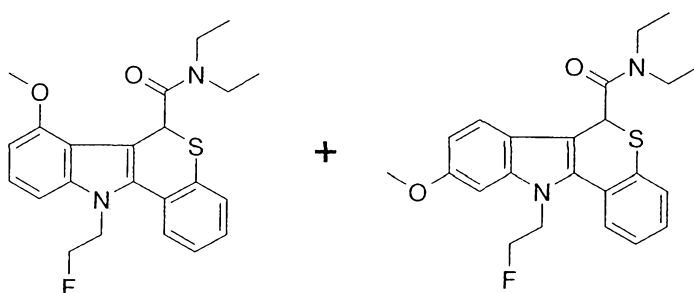
Example 6(i): 7-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic

15   *acid diethylamide and 9-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-*

carboxylic acid diethylamide

To a solution of (+-)-4-Oxo-thiochroman-2-carboxylic acid diethyl amide (3.33 g, 12.6

mmol) (Example 1(i)) and 3-methoxyphenyl hydrazine hydrochloride (2.2 g, 12.6

20   mmol) in ethanol (30 ml) was added concentrated sulphuric acid (1.83 ml, 3.40 g, 11.5

mmol) under nitrogen. The reaction mixture was heated under reflux for 24 h. After

cooling, the reaction mixture was filtered, the solid washed with ethanol, dried *in vacuo*

(45°C) to give a mixture of 7-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-

carboxylic acid diethylamide and 9-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]-

5   fluorene-6-carboxylic acid diethylamide (3.2 g, 69%) as a pale white solid.

Example 6(ii): 11-(2-fluoroethyl)-7-methoxy-6,11-dihydro-5-thia-11-aza-
benzo[a]fluorene-6-carboxylic acid diethylamide and 11-(2-fluoroethyl)-9-methoxy-
6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide

10   To a solution of mixture isomers 7-methoxy-6,11-dihydro-5-thia-11-aza-

benzo[a]fluorene-6-carboxylic acid diethylamide and 9-methoxy-6,11-dihydro-5-thia-

11-aza-benzo[a]-fluorene-6-carboxylic acid diethylamide (1.0 g, 2.73 mmol) (prepared

according to Example 6(i)) in anhydrous DMF (10 ml) was added 2-fluoroethyl tosylate

(1.2 g, 5.46 mmol) followed by sodium hydride 60% dispersion in mineral oil (131 mg,

15   5.46 mmol) under nitrogen. The reaction mixture was heated at 80°C for 1 h. After

cooling, the solvents were removed *in vacuo*, the residue quenched with water (30 ml),

extracted with DCM (2 x 30 ml), dried (MgSO$_4$) and solvents removed *in vacuo*. The

residue was purified by column chromatography on silica, eluting with 5-10%

EtOAc/CH$_2$Cl$_2$ to give the isomer mixture (1.0g, 89%). The material (400 mg) was

20   then purified by HPLC eluting with water (A) and methanol (B) (Gemini 5u, C18,

110A, 150 x 21mm, 70-95% B over 20 min, 21 mL/min) to afford 240 mg of 9-

methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide

as a yellow solid and 100mg of 7-methoxy-6,11-dihydro-5-thia-11-aza-

benzo[a]fluorene-6-carboxylic acid diethylamide as a white solid.

Example 7: Preparation of ^{18}F-labelled Imaging Agents 2 and 7

Example 7(i): 11-[2-(tertbutyl-dimethyl-silanyloxy)]ethyl]-7-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide and 11-[2-(tertbutyl-dimethyl-silanyloxy)]ethyl]-9-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-
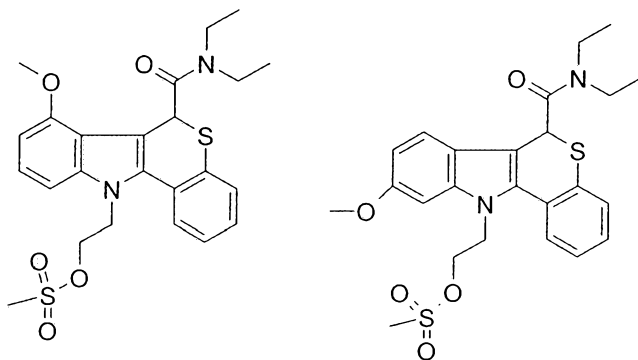5   *carboxylic acid diethylamide*
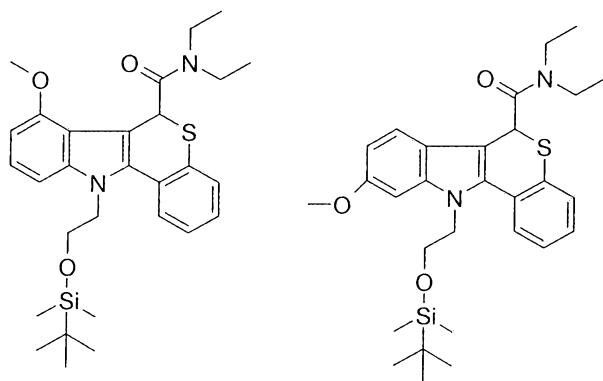
To a solution of the mixture of isomers prepared according to Example 6(i) (2.0 g, 5.46 mmol) in anhydrous DMF (20 ml) was added sodium hydride 60% dispersion in mineral oil (240 mg, 6.0 mmol) and the mixture stirred at room temperature for 5 min

10   under nitrogen. 2-(bromoethoxy)-tert-butyl-dimethylsilane (2.6 g, 10.9 mmol) was added and the mixture stirred for 4h. The solvents were removed *in vacuo*, the residue quenched with water (30 ml), extracted with DCM (2 x 30 ml), dried (MgSO$_4$) and solvents removed *in vacuo*. The residue was purified by column chromatography on silica, eluting with 5% EtOAc/CH$_2$Cl$_2$ to give the isomer mixture 11-[2-(tertbutyl-

15   dimethyl-silanyloxy)]ethyl]-7-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide and 11-[2-(tertbutyl-dimethyl-silanyloxy)]ethyl]-9-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide (2.53g, 88%) as a yellow solid.

Example 7(ii): 11-[2-hydroxyethyl]-7-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide and 11-[2-(2-hydroxyoxy)]ethyl]-7-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide
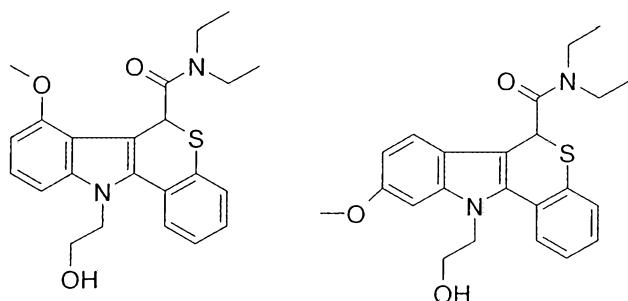
5   To a solution of mixture isomers prepared according to Example 7(i) (2.5 g, 4.76 mmol) in anhydrous THF (40 ml) was added TBAF 1.0 M in THF (9.5 ml, 9.5 mmol) under nitrogen. The mixture was stirred at room temperature for 4 h. The solvents were removed *in vacuo*, the residue was purified by column chromatography on silica, eluting with 40% EtOAc/CH$_2$Cl$_2$ to give the isomer mixture 11-[2-hydroxyethyl]-7-methoxy-

10   6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide and 11-[2-(2-hydroxyoxy)]ethyl]-7-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide (1.90 g, 97 %) as a pale yellow solid.

Example 7(iii): (+-) 11-[2-methanesulphoxyethyl]-7-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide and (+-) 11-2-

15   *(methanesulphoxyethyl)-9-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide*

To a solution of mixture isomers prepared according to Example 7(ii) (1.0 g, 2.4 mmol) dissolved in anhydrous DCM (30 ml) was added pyridine (1.9 g, 24.0 mmol, 1.9 ml). The reaction was cooled to $0°C$ and methane sulfonyl chloride (1.1 g, 9.6 mmol, 0.74 ml) was added. The reaction mixture was stirred at RT for 4h. The mixture was washed

5   with 0.5M HCl (2x20 ml), then water 2x20 ml), dried ($MgSO_4$) and the solvent removed under reduced pressure. The residue was purified by column chromatography on silica, eluting with 20% EtOAc/CH_2Cl_2 to give the isomer mixture (1.0g, 85%). The material (400 mg) was then purified by HPLC eluting with water (A) and methanol (B) (Gemini 5u, C18, 110A, 150 x 21mm, 5-95% B over 30 min, 21 mL/min) to afford

10   170 mg of 11-[2-methanesulphoxyethyl]-7-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide as a white solid and 60mg of 11-2-(methanesulphoxyethyl)-9-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide as a white solid.

Example 7(iv): Direct labeling method

15   The precursor compounds 11-[2-methanesulphoxyethyl]-7-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide and 11-2-(methanesulphoxyethyl)-9-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a]fluorene-6-carboxylic acid diethylamide, prepared according to Example 7(iii) were radiolabelled by a direct labeling method to obtain imaging agents 2 and 7, respectively. ^{18}F water

20   was added to the reaction vessel followed by K222 (2mg) in acetonitrile (500ul), and $KHCO_3$ (0.1mol dm^{-3}, 50ul) and dried at $100°C$ for 20-30mins. The precursor (0.5-1mg) in acetonitrile (1000ul) was added. The reaction vessel was sealed and heated at $100°C$ for 10mins. The reaction mixture was cooled, washed from the reaction vessel with water (1.5ml) and purified on a semi preperative HPLC. The fraction containing

25   the main radioactive product was collected and diluted to a volume of 10ml with H_2O. This was loaded onto a conditioned light C18 sep pak, flushed with H_2O (1x2ml), and the product eluted with EtOH (0.5ml) into a P6 vial and PBS(5ml) was added.

Example 8: Preparation ^{18}F-labelled Imaging Agents 1, 3, 4 and 5

Example 8(i): Preparation of Precursor Compounds

The precursor compounds:

5

(a)   (+-)-8-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a] fluorene-6-
carboxylic acid diethyl amide (prepared according to Example 1(ii));

(b)   (+-)-10-methoxy-6,11-dihydro-5-thia-11-aza-benzo[a] fluorene-6-
carboxylic acid diethyl amide (prepared according to Example 2(i));

(c)   (+-)-4-Fluoro-6,11-dihydro-5-thia-11azabenzo[a]fluorene-6-carboxylic
acid diethylamide (prepared according to Example 3(iii)); and,

10

(d)   (+-) 3-Fluoro-11-(2-fluoro-ethyl)-6,11-dihydro-5-thia-11-aza-
benzo[a]fluorene-6-carboxylic acid diethylamide (prepared according to
Example 4(iv))

were radiolabelled using the indirect labeling method described below to obtain [18]F-
labelled imaging agents 1, 3, 4 and 5, respectively.

15   *Example 8(ii): Indirect labeling method*

[18]F/water was added to K222 (4mg), aqueous K_2CO_3 (50μl of a 0.1 molar solution) and
acetonitrile (500μl) in a reaction vessel and dried for 20-30mins at 100°C under a stream
of nitrogen. Ethyl-1,2-ditosylate (4mg) in acetonitrile (1000ul) was added and heated at
100°C for 10mins. The reaction mixture was cooled and purified by semi preperative

20   HPLC and the fraction containing ^{18}F-fluoroethyl tosylate was collected. This fraction
was diluted to a volume of *ca.*20ml with H_2O, loaded onto a conditioned light t-C18 sep
pak, and flushed with H_2O (1x2ml). The sep pak was dried on the N_2 line with high
flow, for 20mins. The ^{18}F fluoroethyl tosylate was then eluted with DMF(500μl).

Separately, the precursor (13mg) in DMF(250ul) was added to a second reaction vessel,

25   and purged with N_2 for 5mins. NaH(1.3mg) in DMF(2x250ul) was then added under
nitrogen and the reaction vessel was heated at 45°C for 0.5-1h. To this was then added

the ^{18}F fluoroethyl tosylate in DMF prepared above and heated at 100°C for 10mins in the N$_2$ purged reaction vessel. The reaction was cooled and washed from the reaction vessel with water (1ml). The solution was filtered through a syringe filter and purified on a preparative HPLC. The fraction containing the main radioactive peak was

5   collected. This was diluted to a volume of *ca.*10ml with H$_2$O, and loaded onto a conditioned light C18 sep pak, flushed with H$_2$O (1x2ml), and eluted with EtOH (0.5ml) into a P6 vial and Phosphate Buffered Saline (5ml) added.

Example 9: Preparation of ^{18}F-labelled Imaging Agent 6

(+-) 11-[2-methanesulphoxyethyl]-8-ethoxy-6,11-dihydro-5-thia-11-aza-

10   benzo[a]fluorene-6-carboxylic acid diethylamide (prepared according to Example 5(iv)) was radiolabelled using the direct labeling method described in Example 7(iv) above.

Example 10: **In Vitro** *Potency Assay*

The compounds were screened for their affinity for PBR using a method adapted from Le Fur *et al* (Life Sci. 1983; USA 33: 449-57).

15   The compounds to be tested (dissolved in 50mM Tris-HCl, pH 7.4, 10mM MgCl$_2$ containing 1%DMSO) competed for binding to Wistar rat heart PBR against 0.3 nM [^{3}H]-PK-11195. The reaction was carried out in 50mM Tris-HCl, pH 7.4 10mM MgCl$_2$ for 15 minutes at 25°C.

The compounds were screened at 6 different concentrations over a 300-fold range of

20   concentrations around the estimated Ki. The results are presented in Table 1 above, and demonstrate that the potency of the compounds of the invention favourably compares with that of the prior art compounds.

Example 11: **In Vivo** *Biodistribution Method*

In vivo imaging agents 1-7 as prepared in Examples 7, 8 and 9 above were tested in the

25   *in vivo* biodistribution model and their biodistribution compared to that of the prior art compound [^{18}F]FE-PBR (prepared according to Example 14 of WO 2007/057705).

Adult male Wistar rats (200–300g) were injected with 1–3 MBq of each *in vivo* imaging agent via the lateral tail vein. At 2, 10, 30 or 60 min (n = 3) after injection, rats were euthanised and tissues or fluids were sampled for radioactive measurement by liquid scintillation counting.

As compared with [^{18}F]FE-PBR, the compounds of the invention demonstrated a higher olfactory bulb:striatum ratio of binding (see Table 1 above).

Example 12: Autoradiography using Facial Nerve Axotomy (FNA) Model

For *in vivo* studies, male Wistar rats (180–200g) were used. Under Isoflurane anaesthesia, the hair from the right side of the auricular region was removed. An infraauricular incision was made and the main trunk of the facial nerve identified. The facial nerve was severed behind the ear at the exit from the stylomastoid foramen. The wound was sutured and animals left to recover. Seven days post-surgery animals were injected with ~ 5 - 10MBq *in vivo* imaging agent 1 via the lateral tail vein. Animals were killed 30 minutes later and brain stem removed and frozen in isopentane. Cryostat sections (12μm) of brainstem containing both facial nuclei were mounted on glass slides and exposed to phosphor screen overnight.

Screens were then scanned on the Storm (GE Healthcare) phosphor-imager and the resultant scan was analysed and quantified using ImageQuant TL (GE Healthcare). Figures 1 and 2 illustrate the data obtained in this experiment.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An *in vivo* imaging agent of Formula I:
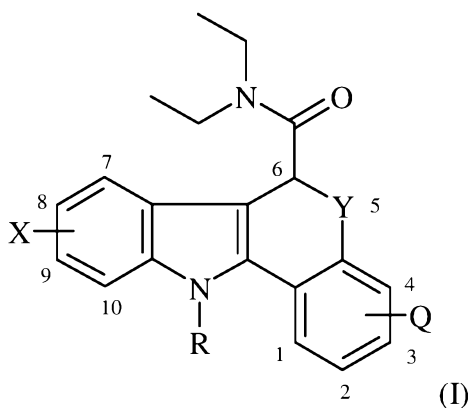
(I)

or a salt or solvate thereof wherein:

Q is hydrogen or fluorine;

X is hydrogen or C_{1-4}alkoxy;

Y is S, and,

R is C_{1-6}fluoroalkyl;

and wherein at least one atom of said *in vivo* imaging agent of Formula I is a radio isotope suitable for *in vivo* imaging which is either a gamma-emitting radioactive halogen or a positron-emitting radioactive non-metal; wherein when said radioisotope is a radio isotope of carbon, it is a carbonyl carbon; with the proviso that Q or X are not both hydrogen.

2. An *in vivo* imaging agent as defined in Claim 1 wherein:

Q is hydrogen; and,

X is C_{1-4}alkoxy.

3. An *in vivo* imaging agent as defined in Claim 1 wherein:
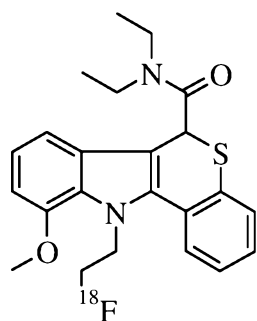
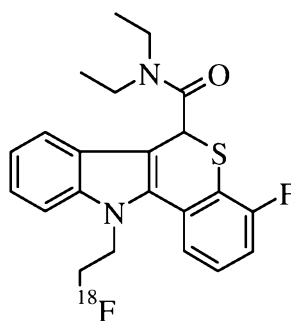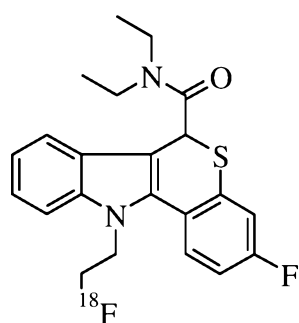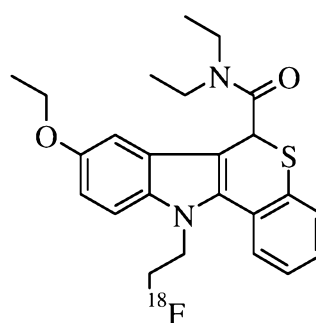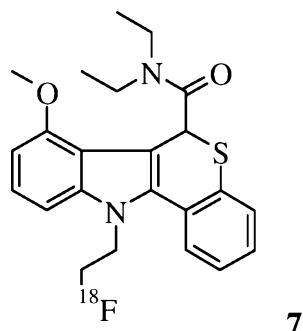Q is fluorine; and,

X is hydrogen

.

4. An *in vivo* imaging agent as defined in any one of claims 1 to 3 wherein said radioisotopeis a positron-emitting radioactive non-metal is ^{18}F.

5. An *in vivo* imaging agent as defined in Claim 4 wherein R is $C_{1-4}[^{18}F]$-fluoroalkyl.

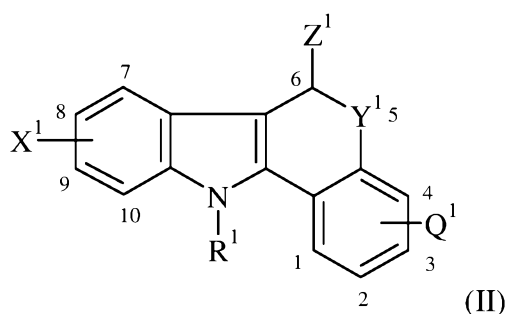6. An *in vivo* imaging agent as defined in Claim 1 which is selected from the group consisting of:

1

2

3

4

5

6

7
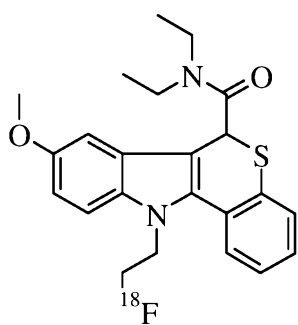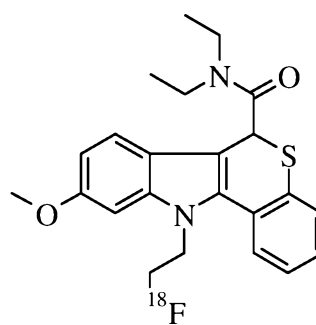
7. A precursor compound useful in the preparation of the *in vivo* imaging agent as defined in any one of Claims 1-6, said precursor compound being of Formula II:

(II)

wherein one of R^1, X^1 or Z^1 comprises a chemical group that reacts with a suitable source of a radioisotope, where said radioisotope is as defined in either Claim 1 or 4, such that said *in vivo* imaging agent is formed upon reaction of said precursor compound with said suitable source of said radioisotope;

and wherein:

when R^1 does not comprise said chemical group it is as defined for R in Claim 1, and optionally further comprises a protecting group;

when X^1 does not comprise said chemical group it is as defined for X in Claim 1-, and optionally further comprises a protecting group;

when Z^1 does not comprise said chemical group it is $-C(=O)-N-(CH_2-CH_3)_2$ and optionally further comprises a protecting group;

Q^1 is as defined for Q in any one of Claims 1-3; and,

Y^1 is as defined for Y in any one of Claims 1-3, and optionally further comprises a protecting group;

with the proviso that Q^1 or X^1 are not both hydrogen.

8. A method for the preparation of the *in vivo* imaging agent as defined in any one of Claims 1-6, said method comprising:

(i)   providing a precursor compound of Formula II as defined in Claim 7;

(ii)   providing a suitable source of said radioisotope, said radioisotope as defined in   either Claim 1 or 4;

(iii)   reacting the precursor compound of step (i) with the source of radioisotope of   step (ii) to obtain said *in vivo* imaging agent.

9. A cassette for the automated synthesis of the *in vivo* imaging agent as defined in any one of Claims 1 to 6, said cassette comprising:

(i) a vessel containing the precursor compound as defined in Claim 7; and

(ii) means for eluting the vessel with a suitable source of a radioisotope, said radioisotope as defined in either Claim 1 or 4.

10. A radiopharmaceutical composition comprising an *in vivo* imaging agent as defined in any one of Claims 1 to 6, together with a biocompatible carrier in a form suitable for mammalian administration.

11. An *in vivo* imaging method for determining the distribution and/or the extent of PBR expression in a subject comprising:

(i)   administering to said subject an *in vivo* imaging agent as defined in any one of   Claims 1 to 6;

(ii)   allowing said *in vivo* imaging agent to bind to PBR in said subject;

(iii)   detecting by an *in vivo* imaging procedure signals emitted by the radio isotope   of said *in vivo* imaging agent;

(iv)   generating an image representative of the location and/or amount of said signals; and,

(v)   determining the distribution and extent of PBR expression in said subject wherein said expression is directly correlated with said signals emitted by said   *in vivo* imaging agent.

12. An *in vivo* imaging method of Claim 11 which is carried out repeatedly during the course of a treatment regimen for said subject, said regimen comprising administration of a drug to combat a PBR condition.

13. A method of diagnosis of a condition in which PBR is up regulated comprising the method of *in vivo* imaging as defined in either of Claims 11 or 12, together with a further step (vi) of attributing the distribution and extent of PBR expression to a particular clinical picture.

14. An *in vivo* imaging agent as defined in any one of Claims 1-6 for use in the method for diagnosis of Claim 13.

15. An *in vivo* imaging agent as defined in any one of Claims 1-6 in the manufacture of a radio pharmaceutical composition as defined in Claim 10 for use in a method of diagnosis as defined in Claim 13.

16. An *in vivo* imaging agent according to claim 1 substantially as hereinbefore described with reference to any one of the Examples and/or Figures.
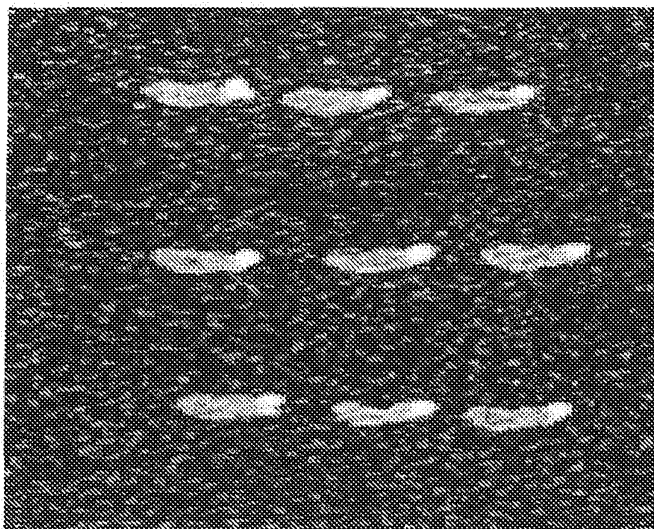
Figure 1: Representative autoradiographs showing binding of *in vivo* **imaging agent 1 in the lesioned (right hand) facial nucleus of the FNA rat.**
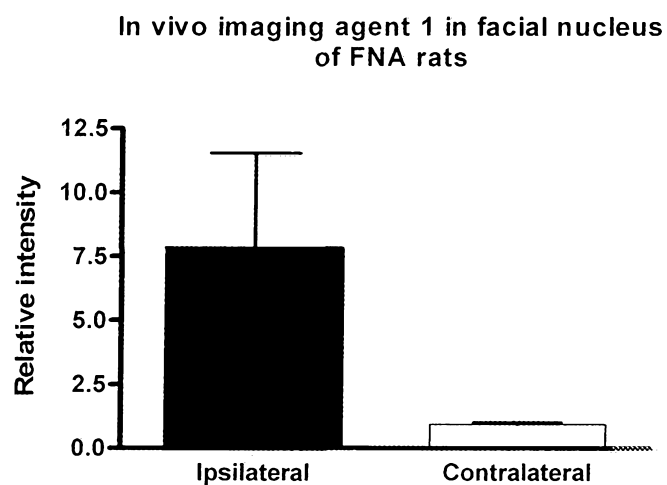
In vivo imaging agent 1 in facial nucleus
of FNA rats

Figure 2: Relative intensity of *in vivo* imaging agent 1 binding in the facial nucleus of a rat seven days post-FNA. Data are expressed as mean ± SD of 24 individual sections from 1 animal.