Title: BENZOTHIAZOLE DERIVATIVES FOR IN VIVO IMAGING OF AMYLOID PLAQUES

Abstract: The invention provides use of a compound of formula (I); or a salt thereof, wherein: R1 is 1H, 2H, 3B, 4B, 5B, or 6B; R2 is C1-6 alkyl; and R3 is selected from hydrogen, C1-6 alkyl, -C(O)C1-6 alkyl, -C(O)C1-6 haloalkyl, and -C(O)CH(R')NH2; wherein R4 is selected from hydrogen, C1-6 alkyl, C1-4 hydroxyalkyl, and C1-4 aminoalkyl; for the manufacture of a radiopharmaceutical for the in vivo diagnosis or imaging of an amyloid-associated disease, particularly Alzheimer’s disease.
BENZOTHIAZOLE DERIVATIVES FOR IN Vivo IMAGING OF AMYLOID PLAQUES

The present invention relates to the field of diagnostic imaging of Alzheimer's disease and provides compounds useful in such diagnostic imaging.

Alzheimer's disease is the fourth most common cause of death in the western world, after heart disease, cancer and strokes. In the USA there are approximately 4 million people suffering with Alzheimer's disease, at an annual cost of $100 billion. Therefore, the cost per person in the USA is $25,000 per year. There are currently 20 million sufferers of dementia in the world. This is set to double to 40 million by the year 2025 as the number of people aged 65 doubles from 390 million now to 800 million in 2025. Of these 40 million, approximately 56 percent will be suffering from Alzheimer's disease, accounting for 22.2 million.

The in vivo imaging techniques used at present do not in all cases differentiate the diagnosis of Alzheimer's disease from other forms of dementia. The differential diagnosis of patients will become increasingly important as more treatments become available. Imaging agents will also be required to image Alzheimer patients at earlier stages of the disease to allow preventive treatment, and for monitoring disease progression.

Currently the only definitive test for Alzheimer's disease is examination of the brain at autopsy for the presence of distinctive pathophysologies. One of the most widely acknowledged of these pathophysologies is the presence of senile plaques in brain tissue. Senile plaques are deposits of a 40-43 amino acid protein called the β-amyloid protein. They are an early and invariant aspect of the disease and it is thought that the deposition of β-amyloid occurs some time prior to the onset of clinical symptoms.

Amyloid-specific radiotracers have been suggested as potential imaging agents for Alzheimer's disease. Congo Red has been demonstrated to be an effective binder of β-amyloid, but does not cross the blood-brain barrier (BBB) well (Klunk et al 1994 Neurobiology of Aging Vol. 15 pp. 691-698). There is no convincing functional evidence that abnormalities in the BBB reliably exist in Alzheimer's.
(Kalaria 1992, Cerebrovascular and Brain Metabolism Reviews, Vol 4, p 226). Therefore, an important property of an Alzheimer's imaging agent is that it crosses the BBB.

WO01/14354 describes a broad class of substituted 2-arylbenzazole compounds and their use as anti-tumour agents.

The aim of the present invention is the provision of novel agents for imaging Alzheimer's disease. To be able to successfully image Alzheimer's disease, an agent must be capable of crossing the BBB as well as binding to β-amyloid.

In a first aspect, this invention provides use of a compound of formula (I):

![Chemical Structure](image)

or a salt thereof, wherein:

R₁ is $^{125}$I, $^{124}$I, $^{123}$I, $^{75}$Br, $^{76}$Br, or $^{18}$F;

R² is C₁₋₆ alkyl; and

R³ is selected from hydrogen, C₁₋₆ alkyl, -C(O) C₁₋₆ alkyl, -C(O)C₁₋₆ haloalkyl, and -C(O)CH(R⁴)NH₂;

wherein R⁴ is selected from hydrogen, C₁₋₆ alkyl, C₁₋₆ hydroxyalkyl, and C₁₋₆ aminoalkyl;

for the manufacture of a radiopharmaceutical for the in vivo diagnosis or imaging of an amyloid-associated disease, particularly Alzheimer's disease.

In a particular aspect, this invention provides use of a compound of formula (I):
or a salt thereof, wherein:

$R^1$ is $^{125}$I, $^{124}$I, $^{123}$I, $^{75}$Br, $^{76}$Br, or $^{18}$F;

$R^2$ is C$_{1-6}$ alkyl; and

$R^3$ is selected from hydrogen, C$_{1-6}$ alkyl, -C(O) C$_{1-6}$ alkyl, and -C(O)C$_{1-6}$ haloalkyl;

for the manufacture of a radiopharmaceutical for the *in vivo* diagnosis or imaging of an amyloid-associated disease, particularly Alzheimer's disease.

In the alternative, there is provided a method for the *in vivo* diagnosis or imaging of amyloid-associated disease in a subject (preferably, a human) comprising administration of a compound of formula (I) or a salt thereof. The method is especially preferred for the *in vivo* diagnosis and imaging of Alzheimer's disease.

"Amyloid-associated" diseases include Alzheimer's disease, familial Alzheimer's disease, type II diabetes, Down's syndrome, homozygotes for the apolipoprotein E4 allele, rheumatoid arthritis, systemic amyloidosis (primary and secondary), and haemorrhagic stroke.

"Alkyl" used either alone or as part of another group (such as haloalkyl) is defined herein as any straight or branched C$_n$H$_{2n+1}$ group, wherein unless otherwise specified n is 1 to 6.

The term "halo" means a group selected from fluoro, chloro, bromo, and iodo.

Suitable salts of the compounds of formula (I) include acid addition salts such as those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric, and sulphuric acids or those derived from organic acids such as tartaric, acetic, trifluoroacetic, citric, malic, lactic, fumaric, benzoic, glycollic, gluconic, succinic, methanesulphonic, and arylsulphonic (for example
para-toluenesulphonic) acids.

In a further aspect of the present invention, there is provided a compound of formula (I) or a salt thereof, for in vivo diagnosis or imaging of amyloid-associated diseases, preferably of Alzheimer’s disease.

A compound of formula (I) or a salt thereof is preferably administered in a radiopharmaceutical formulation comprising the compound of the invention. A “radiopharmaceutical formulation” is defined in the present invention as a formulation comprising compound of formula (I) or a salt thereof in a form suitable for administration to humans, preferably a radiopharmaceutical formulation further comprises a physiologically acceptable excipient. Administration is preferably carried out by injection of the formulation as an aqueous solution. Such a formulation may optionally contain further ingredients such as buffers; pharmaceutically acceptable solubilisers (e.g. cyclodextrins or surfactants such as Pluronic, Tween or phospholipids); pharmaceutically acceptable stabilisers or antioxidants (such as ascorbic acid, gentisic acid or para-aminobenzoic acid). The dose of a compound of formula (I) or a salt thereof will vary depending on the exact compound to be administered, the weight of the patient, and other variables as would be apparent to a physician skilled in the art. Generally, the dose would lie in the range 0.001 µg/kg to 10 µg/kg, preferably 0.01 µg/kg to 1.0 µg/kg.

In a particular aspect of the invention, in the compound of formula (I), \( R^1 \) is \(^{123}\text{I} \). Such compounds are useful for SPECT imaging of amyloid-associated diseases, such as Alzheimer’s disease.

In another particular aspect of the invention, in the compound of formula (I), \( R^1 \) is \(^{125}\text{I} \). Such compounds are useful for SPECT imaging of amyloid-associated diseases, such as Alzheimer’s disease.

In another particular aspect of the invention, in the compound of formula (I), \( R^1 \) is \(^{18}\text{F} \). Such compounds are useful for Positron Emission Tomography (PET) imaging of amyloid-associated diseases, such as Alzheimer’s disease.
In another particular aspect of the invention, in the compound of formula (I), R$^1$ is $^{124}$I. Such compounds are useful for PET imaging of amyloid-associated diseases, such as Alzheimer’s disease.

Preferred compounds of formula (I) include those in which:

R$^1$ is $^{125}$I, $^{124}$I, $^{123}$I or $^{18}$F;

R$^2$ is methyl; and

R$^3$ is selected from hydrogen and -C(O)C$_{1-6}$ haloalkyl, suitably -C(O)C$_{1-6}$ fluoroalkyl, most suitably -C(O)CF$_3$.

Where R$^3$ is -C(O)CH(R$^4$)NH$_2$, R$^4$ is preferably C$_{1-6}$ aminoalkyl, and is more preferably -(CH$_2$)$_n$NH$_2$. One such compound of particular interest is 5-$^{18}$F-fluoro-2-(4'-amino-3'-methylphenyl)benzothiazole lysyl amide or a salt thereof such as the dihydrochloride salt.

Particularly preferred compounds of formula (I) include:

5-$^{125}$I-iodo-2-(4'-amino-3'-methylphenyl)benzothiazole;

5-$^{125}$I-iodo-2-(4'-trifluoromethylamido-3'-methylphenyl)benzothiazole;

5-$^{18}$F-fluoro-2-(4'-amino-3'-methylphenyl)benzothiazole;

5-$^{18}$F-fluoro-2-(4'-trifluoromethylamido-3'-methylphenyl)benzothiazole;

or a salt thereof.

Certain of the compounds of formula (I) are novel, and therefore, as a separate aspect of the invention there is provided a compound of formula (Ia):

![Chemical Structure](image)
or a salt thereof, wherein:

R\(^1\) is \(^{125}\text{I}, ^{124}\text{I}, ^{123}\text{I}, ^{75}\text{Br}, \text{ or } ^{76}\text{Br};

R\(^2\) is C\(_{1-6}\) alkyl; and

R\(^3\) is selected from hydrogen, C\(_{1-6}\) alkyl, -C(O) C\(_{1-6}\) alkyl, and -C(O)C\(_{1-6}\) haloalkyl.

Compounds of formula (I) may be prepared by iodination, bromination or fluorination of the corresponding precursor compound in which R\(^1\) is a tri(C\(_{1-6}\)alkyl)tin substituent, suitably a trimethyltin substituent. These precursors may be prepared according to the methods described in WO 01/14354 (in particular, Example 44 thereof). The iodination reaction may be effected using an iodide source, such as sodium iodide, in the presence of an oxidising agent, suitably N-chlorosuccinimide, an N-chlorotolylsulphonamide (for example, chloramine T or iodogen), or peracetic acid at non-extreme temperature, preferably at ambient temperature, and in a suitable solvent such as an aqueous buffer at pH 6 to 8, preferably pH 7.4. The fluorination reaction may be effected using the methods described in WO 01/14354 (in particular, Example 45 thereof). The bromination reaction may be effected using a bromide source, such as sodium bromide, in the presence of an oxidising agent, suitably N-chlorosuccinimide, an N-chlorotolylsulphonamide (for example, chloramine T or iodogen), or peracetic acid at non-extreme temperature, preferably at ambient temperature, and in a suitable solvent such as an aqueous buffer.

The \([^{18}\text{F}]-\)fluorinated compounds of formula (I) may also be prepared using the solid-phase fluorination methods described in WO 03/002157 and WO 03/002489.

The invention will now be illustrated by way of the following Examples.

**Example 1: Synthesis of 5-[\(^{125}\text{I}]-\)iodo-2-(4'-amino-3'-methylphenyl)benzothiazole (Compound 1)**

The title compound is prepared from Compound 2 (Example 2) by treatment with potassium hydroxide by a method analogous to that described in Example 3.
Example 2: Synthesis of 5-[125I]-ido-2-(4'-trifluoromethylamido-3'-methylphenyl)benzothiazole (Compound 2)

To 5-trimethylstannyl-2-(4'-trifluoromethylamido-3'-methylphenyl)benzothiazole (which may be prepared as described in WO 01/14354) (100μg), was added 300μl sodium phosphate buffer pH7.4, together with 10mCi [125I]-sodium iodide and 100μl of chloramine T (1mg/ml in water). This mixture was allowed to react for 30 seconds and the reaction was terminated by adding 100μl sodium metabisulphite. The mixture was loaded onto a C4 column and separated on reverse phase HPLC using eluents A=water + 0.1% trifluoroacetic acid (TFA), B=acetonitrile + 0.1% TFA. The product was collected and diluted to 250μCi/ml in methanol solution and stored at 4°C.

HPLC QC analysis showed the product to have a Radiochemical purity (RCP) of 93% and Iodide content of 0.5%

Example 3: Synthesis of 5-[18F]-fluoro-2-(4'-amino-3'-methylphenyl)benzothiazole (Compound 3)

Fluorine-18, produced as gaseous molecular fluorine (18F-F) by the 18O(p, n)18F nuclear reaction was bubbled through a solution of 5-trimethylstannyl-2-(4'-trifluoromethylamido-3'-methylphenyl)benzothiazole (which may be prepared as described in WO 01/14354) (20 mg, 40 μmol) in acetonitrile (10 mL) and the solvent was removed under reduced pressure. The residue was dissolved in ethanol (1 mL) and potassium hydroxide (1 mL, 0.2 M) and then heated at 80-90°C for 10 minutes. The resultant mixture was loaded onto an HPLC column (μ-Bondapak C18, 30 x 0.78 cm i.d.) eluted with a mixture of acetonitrile:water (55:45) at a flow rate of 3 mL min⁻¹. The eluent was monitored for radioactivity and UV absorbance at 254 nm. The radioactive peak having the same retention time of 12-14 min was collected. The eluent was removed under reduced pressure to yield the title compound.

Example 4: Synthesis of 5-[18F]-fluoro-2-(4'-trifluoromethylamido-3'-methylphenyl)benzothiazole (Compound 4)

Fluorine-18, either as gaseous molecular fluorine (18F-F) or [18F]acetyl hypofluorite
is bubbled through a solution of 5-trimethylstannyl-2-(4'-trifluoromethylamido-3'-methylphenyl)benzothiazole (20 mg, 40 μmol) in acetonitrile (10 mL) and the solvent removed under reduced pressure. Compound 4 is isolated in a pure form using column chromatography.

5 Biological Data

A. Blood Brain Barrier (BBB) Permeability

Culturing of CACO-2 cells and determination of Apparent Permeability (Papp) values:

CACO-2 cells (ATCC number HTB-37) derived from colorectal adenocarcinoma in a 72 year old male were initially cultured in 75 cm² cell culture flasks (Costar 3376) until confluent. CACO-2 cells were grown in EMEM (Sigma 4526) containing 10% FCS, 10 μg/ml insulin (HYBRI-MAX, Sigma I-4011), non-essential amino acids (Sigma, M7145), glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin (Sigma, P0906). All cells were incubated at 37°C in 95% air/5% CO₂. At confluence the cells were used to seed 12 mm Transwell-Col inserts (Costar 3493).

The seeding of 12 mm Transwells was as follows for CACO-2. Flasks of confluent cultures were trypsinised and cells were carefully resuspended, making sure there are no clumps or air bubbles. 1.5 ml of tissue culture medium was placed in the bottom (acceptor) chamber of the wells and 0.5 ml containing 2 x 10⁶ cells in the Transwell (donor chamber) and placed in the incubator. The cells were routinely monitored for adequate trans epithelial electrical resistance (TEER) using an EndOhm Tissue resistance measurement chamber (WPI). The subsequent maintenance and feeding of the cells on the Transwells was as follows: when feeding the wells, the medium was removed from the acceptor chamber (basolateral side) and donor chamber (apical side) of the Transwells. The medium was aspirated off with a pipette connected to a vacuum pump, being careful not to touch the filter. 0.5 ml of growth medium was then placed into the donor chamber and 1.5 ml of growth medium was placed into the acceptor chamber.

When the TEER values of the CACO-2 cell monolayers in Transwells was around 500 Ωcm² (300-800 Ωcm² was considered acceptable) permeability experiments
were performed in triplicate as follows. All culture media was removed and acceptor and donor chambers were rinsed twice with Eagles Balance Salt Solution (EBSS) (Gibco) at 37°C. 1.5 ml EBSS was added to the acceptor chamber and 0.5 ml EBSS containing radiolabelled compound was added to the donor chamber. Cultures in Transwells were then incubated for 30 minutes at 37°C at 200 rpm using a Labnet Vortemp. After 30 minutes, 100 µl aliquot was taken from the donor chamber and 750 µl from the acceptor chamber. These aliquots were then counted for radioactivity. The remaining EBSS was removed from the acceptor and donor chambers and then the Transwell was thoroughly rinsed three times with EBSS. Next, the Transwell membrane (and associated cells) was removed using a scalpel and the amount of radiolabel associated with it was determined. Permeability of compounds were determined by calculating their Papp value:

\[ \text{Papp} = \frac{\Delta Q}{\Delta t} \times A \times \text{Co} \quad (\text{cm/sec}) \]

Where, \( \Delta Q/\Delta t \) is the permeability rate (µg/min); Co is the initial concentration of radiolabelled compound; A is the surface area of membrane. The amount of labelled compound present could be determined from the specific activity of the compound (74 TBq \( ^{125}\text{I} \)/mmol).

Results and Discussion

Permeability through the BBB can be either by passive diffusion which requires a compound being small (<500 Da) and Lipophilic. In this assay, permeable compounds have a Papp value of more than \( 1 \times 10^{-5} \). For example, as Table 1 shows, \(^{14}\text{C}-\text{glucose} \) (Amersham Biosciences) which relies on active transport has a Papp of \( 5.79 \times 10^{-6} \), and \(^{14}\text{C}-\text{diazepam} \) (Amersham Biosciences) which relies on being small and lipophilic has a Papp of \( 2.44 \times 10^{-5} \). \(^{14}\text{C}\)-Sucrose (Amersham Biosciences) and \(^{14}\text{C}\)-mannitol (Amersham Biosciences) on the other hand, have Papp values of \( 4.08 \times 10^{-6} \) and \( 3.63 \times 10^{-6} \) respectively, showing that charge is a contributor to impermeability as both these molecules are small. Compound 1 has a Papp value of \( 1.37 \times 10^{-5} \) implying that it is permeable through the CACO-2 cell BBB model used. Compound 1 is relatively lipophilic (LogP of 1.75) and small (462.34 Da) and is not prone to H-bond formation (\( \Delta \text{LogP} \) value of \( -0.38 \) which
also is a benefit when crossing the CACO-2 cell barrier or the BBB. These data suggest that Compound 1 passes through the cell barrier by passive diffusion.

Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Papp (cm sec(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 1</td>
<td>1.37 \times 10^{-5}</td>
</tr>
<tr>
<td>(^{14})C-Glucose</td>
<td>5.79 \times 10^{-5}</td>
</tr>
<tr>
<td>(^{14})C-diazepam</td>
<td>2.44 \times 10^{-5}</td>
</tr>
<tr>
<td>(^{14})C-sucrose</td>
<td>4.08 \times 10^{-6}</td>
</tr>
<tr>
<td>(^{14})C-mannitol</td>
<td>3.63 \times 10^{-6}</td>
</tr>
</tbody>
</table>

5 B. Brain Uptake Index (BUI)

Method

The method used is that used by Cornford et al. “Metaphalan penetration of the blood-brain barrier via the neutral amino acid transporter in tumour bearing brain.” Cancer Res 52 p138-143 (1992) and involves the injection of a bolus of activity directly into the carotid artery. The animals are decapitated after 15 seconds, the brain removed and the uptake of the test compound calculated with reference to a freely diffusable standard (\(^{14}\)C-Butanol, Amersham Biosciences).

BUI is calculated according to the following equation:

\[
BUI(\%) = \frac{cpm \ brain_{\text{test}}}{cpm \ brain_{\text{standard}}} \times 100\% \\
\frac{cpm \ injectate_{\text{test}}}{cpm \ injectate_{\text{standard}}}
\]

BUI was performed on three animals from the same test solution for each experiment. Each compound was assayed in duplicate.

Ceretec\(^{TM}\) and Datscan\(^{TM}\) were obtained from Amersham Health, \(^{14}\)C-FDG and \(^{14}\)C-sucrose were obtained from Amersham Biosciences.
Results

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean BUI</th>
<th>S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceretec</td>
<td>107.25</td>
<td>21.02</td>
</tr>
<tr>
<td>Datscan</td>
<td>73.59</td>
<td>26.50</td>
</tr>
<tr>
<td>Compound 1</td>
<td>43.6</td>
<td>16</td>
</tr>
<tr>
<td>$^{14}$C-FDG</td>
<td>17.98</td>
<td>4.65</td>
</tr>
<tr>
<td>$^{14}$C-Sucrose</td>
<td>0.54</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Conclusion:

It can be seen that the BUI of Compound 1 is comparable to other compounds that cross the blood-brain barrier. The cut off between compounds that are of low CNS penetration and those that show medium BBB penetration is 20%. It is felt that a value of greater than 20% is acceptable BBB penetration for an imaging agent. Compound 1 therefore shows medium to high BBB penetration, indicative of adequate delivery for a diagnostic of Alzheimer's Disease.

10 In Situ Brain Perfusion

Method

Compounds may penetrate the brain slowly or be subject to peripheral metabolism, meaning that the BUI may not always be reflective of BBB permeability. For this reason an in situ brain perfusion technique was used. This technique has been extensively used to evaluate the BBB permeability of compounds. The method used is described by Williams et al. “Passage of a delta-opioid receptor selective enkephalin, [D-penicillamine2,5] enkephalin, across the blood-brain and the blood-cerebrospinal fluid barriers.” J Neurochem. 1996 Mar;66(3):1289-99.

The brain was perfused with a saline-based solution containing the test compound for 2, 5, 15, 20 and 30 minutes and uptake in the brain calculated as a percentage of concentration of test substance in the perfusate (R tissue = dpm per g brain/ dpm per µl perfusate x 100%).

Uptake is plotted against time and the slope of the graph represents the unidirectional brain influx constant, $K_{in}$. This reflects the rate of entry of a compound into the CNS.
Results

<table>
<thead>
<tr>
<th>Compound</th>
<th>Kin (ml/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{14}$C-Sucrose</td>
<td>0.7</td>
</tr>
<tr>
<td>Ceretec</td>
<td>19.8</td>
</tr>
<tr>
<td>Compound 1</td>
<td>49.8</td>
</tr>
</tbody>
</table>

Conclusion

Compound 1 gives a high $K_{in}$ value. This implies that the compound rapidly penetrates the CNS, and backs up the high BUJ value.

Capillary Depletion

Compounds that may appear to cross the blood-brain barrier may be sticking to the vasculature within the brain, rather than entering the brain parenchyma. For this reason a capillary depletion method was employed that separates the capillaries from brain homogenate.

Method:


Male Wistar rats weighing 150-250g were deeply anaesthetised by the administration of 55mg/kg sodium pentabarbital (Rhone-Meriaux) by intra peritoneal injection. The left common carotid artery was exposed by blunt dissection, and 100μl of test solution injected in a single bolus into the carotid using a 30G needle and 1ml syringe (injection time ~1 second). The injectate contained both the test/validation compound and $^{14}$C- or $^{3}$H-Sucrose (Amersham Biosciences) as a non-diffusible standard. Ten seconds after injection, the animal was decapitated and the brain removed. The cerebellum was removed, and the
cortex dissected into the left and right hemispheres. Both hemispheres were weighed before homogenisation in 3.5 times their weight of Hanks Buffered Salt Solution (HBSS) using 10-15 strokes using a glass homogeniser. 4 times the brain weight of 26% Dextran solution (73000 molecular weight dextran in HBSS) was added, and the brain further homogenised using 3-5 strokes with the glass homogeniser. All homogenisation periods were performed at 4°C and were completed within 1 minute. The homogenates were centrifuged for 15 minutes at 5400g at 4°C in a refrigerated centrifuge. The vascular enriched pellet and supernatant were then carefully separated. Pellet, supernatant and a sample of injection solution were counted on a Rackbeta Excel scintillation counter, after the addition of 10ml Hionic Fluor scintillant (Packard). ¹⁴C/³H-Sucrose served to correct for vascular volume of vessels contained in the pellet, and also activity that had leaked from the vasculature into the supernatant during the homogenisation procedure.

Volumes of distribution for pellet and supernatant were calculated as shown below. Data is expressed as ratio of volume of distribution in the supernatant to pellet.

\[ V_D = \frac{\text{DPM test (tissue)}}{\text{DPM test (tissue)}} - \frac{\text{DPM sucrose (injection solution)}}{\text{DPM sucrose (injection solution)}} \]

**Results**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ratio of V_D supernatant/pellet</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹⁴C-Butanol</td>
<td>51.0</td>
</tr>
<tr>
<td>Compound 1</td>
<td>7.2</td>
</tr>
</tbody>
</table>

**Conclusion:**
The results imply that Compound 1 is mainly contained within the brain parenchyma. A proportion may be contained within the vasculature (the ratio of
supernatant to pellet is not as high as a freely diffusable compound such as $^{14}$C-butanol).

From this it can be concluded that Compound 1 is crossing the blood-brain barrier to enter the brain parenchyma, where it would be able to interact with Alzheimer’s Disease pathology. The amount of radiolabel crossing the blood-brain barrier is sufficient to bind and image Alzheimer’s pathology

C. Amyloid Binding

Determination of amyloid binding of Compound 1:

The binding of Compound 1 (74 TBq/mmol) was determined compared to the ability of $^{125}$I-beta amyloid protein 1-40 ($^{125}$I-BAP 1-40, Amersham Biosciences IM294)) to bind to amyloid 1-40 fibrils. Amyloid binding was essentially performed as follows.

Three fresh buffer stocks were prepared for experiments: Buffer 1, 50 mM HEPES/0.1% Bovine Serum Albumin (BSA) pH 7.5; Buffer 2, 50 mM HEPES/0.1% BSA/400 μM ZnCl$_2$ pH 7.5; Buffer 3, 50 mM HEPES/0.1% BSA/100 μM ZnCl$_2$ pH 7.5.

Streptavidin coated scintillation proximity assay beads (SA-SPA beads, Amersham Biosciences) were used to immobilise fibrillar Beta-Amyloid Protein (BAP 1-40).

Amyloid coated beads (SPA-BAP) were prepared by incubating 250 μl SA-SPA beads (100 mg/ml) with 250 μl Buffer 2, 425 μl Buffer 1, 50 μl biotinylated BAP 1-40 (0.5 mg/ml, Biosource 03-243), 25 μl BAP 1-40 (10 mg/ml, Biosource 03-138). Non-specific binding SPA beads (SPA-NSB) were prepared to assess the binding of compounds to SPA beads with no associated BAP 1-40 fibrils in the following incubation: 250 μl SA-SPA beads (100 mg/ml) with 250 μl Buffer 2, 500 μl Buffer 1.

SPA-BAP and SPA-NSB incubations were left for 24 hours at room temperature and then spun 1.5 ml tubes (eppendorf, Merk, 306/0421/12) for 2 minutes at 1000 x g. The supernatants were removed and the beads were washed twice by resuspending them in 1 ml Buffer 3 followed by centrifugation for 2 minutes at
1000 x g. Finally, washed SPA-BAP and SPA-NSB beads were resuspended in 1 ml Buffer 3.

Amyloid binding of $^{125}$I-BAP 1-40 and Compound 1 was performed in triplicate in 0.5 ml tubes (eppendorf, Merk, 306/0421/02) by adding 50 µl SPA-BAP beads to 25 µl Buffer 2 and 25 µl labelled compound ($^{125}$I-BAP 1-40 or Compound 1). Tubes were then incubated for 180 minutes at room temperature with shaking, followed by centrifugation for 2 minutes at 1000 x g. The supernatants were removed and SPA-BAP pellet washed twice with 300 µl Buffer 3 containing 1% TWEEN-20 (Sigma, P7949). Non-specific binding for labelled compounds to the SPA beads was determined using incubations as described above but by substituting SPA-BAP beads with SPA-NSB beads. Radioactivity associated with the washed SPA bead pellets was then determined.

The affinity of labelled compounds for fibrillar BAP 1-40 was estimated by subtracting SPA-NSB associated counts from SPA-BAP associated counts. The binding of labelled compounds was then compared to $^{125}$I-BAP binding, which was taken as being 100%.

In these experiments, $^{125}$I-BAP 1-40 or Compound 1 were added in equimolar amounts (between 1-5 x 10^{-11} mmoles per incubation).

Results and discussion

BAP 1-40 readily self-aggregates. In this assay, the binding of $^{125}$I-BAP 1-40 to a fixed amount of amyloid fibrils immobilised on SPA beads was used as a reference for other compounds. Table 1 shows how other amyloid binders and non-binders compare to the binding of $^{125}$I-BAP 1-40. Compound 1 binds to amyloid fibrils with 27% affinity of $^{125}$I-BAP 1-40, which is favourable compared to the $^{125}$I-labelled BAP 15-21 sequence (Amersham Biosciences) (21%) and the $^{99m}$Tc-labelled BAP 15-21 sequence (9%). The BAP 15-21 sequence is responsible for the binding of BAP to itself during the formation of amyloid fibrils.
Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amyloid binding (% of 125I-BAP 1-40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125I-BAP 1-40</td>
<td>100</td>
</tr>
<tr>
<td>Compound 1</td>
<td>27</td>
</tr>
<tr>
<td>125I-KKLVFFA (BAP 15-21)</td>
<td>21</td>
</tr>
<tr>
<td>99mTc-Pn216-KKLVFF (BAP 15-20)</td>
<td>9</td>
</tr>
</tbody>
</table>

D. Pharmacokinetics

Materials and Method

Compound 1 IMQ1961 prepared by Amersham Biosciences 250 μCi/ml (specific activity 2000Ci/mmol) freshly diluted to give 1μCi/0.1 ml volume injection bolus. 15 normal male wistar rats 150-180g (Charles River).

Male wistar rats were anaethetised briefly prior to injection with Compound 1 (1μCi, 0.1ml bolus tail vein). Biodistributions were performed at five time points (5, 15, 30, 60 and 120 mins) in triplicate animals in which tissues from brain, blood, muscle, kidney, spleen, stomach, small intestines, large intestines and faeces, bladder and urine, fat, skin and thyroid were dissected and counted for distribution of the compound in the body in a Wallac Wizzard gamma counter.

Percentage injected dose calculations were made and plotted using the Qk pharmacokinetics program to determine t½ α, and volume of distribution (V₀). Relative retention (RR) and Brain:tissue ratios were calculated as follows:

Relative retention = \( \frac{\text{cpm brain/brain weight (g)}}{\text{cpm remaining in body/body weight (g)}} \)
Brain:Blood ratio = \frac{cpm \text{ brain/brain weight (g)}}{cpm \text{ blood/blood weight (g)}}

Results and discussion

Biodistribution data are shown for Compound 1 (Table 2 and Figure 1). Data show percentage injected dose in the brain in normal wistar rats changes over the two hour experiment. Initial delivery is 0.94% to the brain which clears out with a t1/2 values of 12.4 min (α) to yield an uptake of 0.29% at 60 mins. Low thyroid uptake (<1%) indicates that the iodinated compound is very stable in vivo and does not degrade to iodide as often the case with other iodinated molecules.

The volume of distribution of the compound is high (2.27 x10^4 L/kg) which is expected for a lipophilic compound such as Compound 1. Compound 1 Log P and delta LogP values of 1.75 and -0.38, respectively facilitate its transport through plasma membranes. Consequently high % ID values in fat tissue and to a lesser extent, skin, are observed.

Relative retention of the compound is the retention in the brain tissue with respect to the rest of the body. Figure 2 shows relative retention for Compound 1 is 1.01 initially at 5 minutes, compared to blood that has a similar 1.04. Decreases in relative retention with time suggest Compound 1 is taken up maximally within five minutes and is then cleared more quickly than clearance through the rest of the tissues in the body. Brain:blood ratios (Figure 3) indicate Compound 1 clears from blood faster than brain between 15 and 30 mins. Clearance through the body is largely via the hepatobiliary system, in keeping with the pharmacokinetics profile for a lipophilic compound and little is via the urinary system.
Table 2

<table>
<thead>
<tr>
<th></th>
<th>5 min SD</th>
<th>5 min SD</th>
<th>15 min SD</th>
<th>15 min SD</th>
<th>30 min SD</th>
<th>30 min SD</th>
<th>60 min SD</th>
<th>60 min SD</th>
<th>120 min SD</th>
<th>120 min SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>21.49</td>
<td>4.00</td>
<td>19.83</td>
<td>1.71</td>
<td>12.59</td>
<td>2.45</td>
<td>7.79</td>
<td>2.17</td>
<td>5.60</td>
<td>0.68</td>
</tr>
<tr>
<td>Blood</td>
<td>6.05</td>
<td>1.58</td>
<td>4.14</td>
<td>0.32</td>
<td>3.58</td>
<td>0.59</td>
<td>2.77</td>
<td>0.35</td>
<td>2.27</td>
<td>0.58</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.14</td>
<td>0.65</td>
<td>1.60</td>
<td>0.22</td>
<td>0.95</td>
<td>0.22</td>
<td>0.63</td>
<td>0.15</td>
<td>0.45</td>
<td>0.14</td>
</tr>
<tr>
<td>urine</td>
<td>0.04</td>
<td>0.01</td>
<td>2.10</td>
<td>2.90</td>
<td>1.63</td>
<td>0.37</td>
<td>8.39</td>
<td>1.96</td>
<td>10.02</td>
<td>1.06</td>
</tr>
<tr>
<td>Lung</td>
<td>1.40</td>
<td>0.26</td>
<td>0.49</td>
<td>0.43</td>
<td>0.48</td>
<td>0.04</td>
<td>0.34</td>
<td>0.11</td>
<td>0.23</td>
<td>0.04</td>
</tr>
<tr>
<td>Liver</td>
<td>19.47</td>
<td>4.01</td>
<td>7.48</td>
<td>2.24</td>
<td>4.46</td>
<td>0.76</td>
<td>3.12</td>
<td>0.40</td>
<td>2.39</td>
<td>0.66</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.69</td>
<td>0.34</td>
<td>0.32</td>
<td>0.07</td>
<td>0.19</td>
<td>0.05</td>
<td>0.15</td>
<td>0.05</td>
<td>0.08</td>
<td>0.01</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.82</td>
<td>1.12</td>
<td>2.56</td>
<td>1.00</td>
<td>6.66</td>
<td>1.60</td>
<td>8.98</td>
<td>1.01</td>
<td>9.29</td>
<td>3.27</td>
</tr>
<tr>
<td>SI</td>
<td>9.04</td>
<td>0.37</td>
<td>12.60</td>
<td>1.83</td>
<td>21.93</td>
<td>2.56</td>
<td>28.42</td>
<td>10.16</td>
<td>26.86</td>
<td>6.27</td>
</tr>
<tr>
<td>LI</td>
<td>1.53</td>
<td>0.77</td>
<td>1.77</td>
<td>1.26</td>
<td>1.90</td>
<td>1.15</td>
<td>7.75</td>
<td>11.99</td>
<td>3.12</td>
<td>3.16</td>
</tr>
<tr>
<td>Brain</td>
<td>0.94</td>
<td>0.14</td>
<td>0.75</td>
<td>0.05</td>
<td>0.49</td>
<td>0.09</td>
<td>0.29</td>
<td>0.07</td>
<td>0.18</td>
<td>0.06</td>
</tr>
<tr>
<td>thyroid</td>
<td>0.16</td>
<td>0.14</td>
<td>0.16</td>
<td>0.12</td>
<td>0.39</td>
<td>0.53</td>
<td>0.17</td>
<td>0.20</td>
<td>0.95</td>
<td>0.52</td>
</tr>
<tr>
<td>Skin</td>
<td>8.92</td>
<td>2.20</td>
<td>12.80</td>
<td>0.33</td>
<td>16.90</td>
<td>1.77</td>
<td>17.14</td>
<td>3.37</td>
<td>12.89</td>
<td>1.05</td>
</tr>
<tr>
<td>Fat</td>
<td>30.82</td>
<td>15.61</td>
<td>36.31</td>
<td>2.86</td>
<td>59.02</td>
<td>13.66</td>
<td>73.92</td>
<td>9.36</td>
<td>55.62</td>
<td>21.14</td>
</tr>
<tr>
<td>inj site</td>
<td>2.12</td>
<td>0.64</td>
<td>1.86</td>
<td>0.58</td>
<td>3.05</td>
<td>2.69</td>
<td>1.83</td>
<td>0.49</td>
<td>2.62</td>
<td>1.59</td>
</tr>
</tbody>
</table>
Claims

1. Use of a compound of formula (I):

\[
\text{R}^1 \text{H}_2 \text{N} \text{R}^3
\]

or a salt thereof, wherein:

\[ R^1 \text{ is }^{125}\text{I}, ^{124}\text{I}, ^{123}\text{I}, ^{75}\text{Br}, ^{78}\text{Br}, \text{ or } ^{18}\text{F}; \]

\[ R^2 \text{ is C}_{1-6} \text{ alkyl; and} \]

\[ R^3 \text{ is selected from hydrogen, C}_{1-6} \text{ alkyl, } -\text{C(O)C}_{1-6} \text{ alkyl, } -\text{C(O)C}_{1-6} \text{ haloalkyl, and } -\text{C(O)CH(R}^4\text{)NH}_2; \]

\[ \text{wherein } R^4 \text{ is selected from hydrogen, C}_{1-6} \text{alkyl, C}_{1-6} \text{hydroxyalkyl, and C}_{1-6} \text{aminoalkyl;} \]

for the manufacture of a radiopharmaceutical for the \textit{in vivo} diagnosis or imaging of an amyloid-associated disease, particularly Alzheimer's disease.

2. Use according to claim 1 of a compound of formula (I):

\[
\text{R}^1 \text{H}_2 \text{N} \text{R}^3
\]

or a salt thereof, wherein:

\[ R^1 \text{ is }^{125}\text{I}, ^{124}\text{I}, ^{123}\text{I}, ^{75}\text{Br}, ^{76}\text{Br}, \text{ or } ^{18}\text{F}; \]

\[ R^2 \text{ is C}_{1-6} \text{ alkyl; and} \]

\[ R^3 \text{ is selected from hydrogen, C}_{1-6} \text{ alkyl, } -\text{C(O)C}_{1-6} \text{ alkyl, and } -\text{C(O)C}_{1-6} \text{ haloalkyl;} \]
for the manufacture of a radiopharmaceutical for the *in vivo* diagnosis or imaging of an amyloid-associated disease, particularly Alzheimer’s disease.

3. Use according to claim 1 or 2, where in the compound of formula (I):

\[
\begin{align*}
R^1 & \text{ is } ^{125}\text{I, }^{124}\text{I, }^{123}\text{I, or }^{18}\text{F;} \\
R^2 & \text{ is methyl;} \quad \text{and} \\
R^3 & \text{ is selected from hydrogen and } -\text{C(O)C}_{1,6} \text{ haloalkyl.}
\end{align*}
\]

4. Use according to any one of claims 1 to 3 wherein the compound of formula (I) is selected from:

\[
\begin{align*}
5-[^{125}\text{I}]-\text{i} & \text{odo-2-(4'}\text{'-amino-3'}\text{'-methylphenyl)benzothiazole;} \\
5-[^{124}\text{I}]-\text{i} & \text{odo-2-(4'}\text{'-amino-3'}\text{'-methylphenyl)benzothiazole;} \\
5-[^{123}\text{I}]-\text{i} & \text{odo-2-(4'}\text{'-amino-3'}\text{'-methylphenyl)benzothiazole;} \\
5-[^{125}\text{I}]-\text{i} & \text{odo-2-(4'}\text{'-trifluoromethylamido-3'}\text{'-methylphenyl)benzothiazole;} \\
5-[^{124}\text{I}]-\text{i} & \text{odo-2-(4'}\text{'-trifluoromethylamido-3'}\text{'-methylphenyl)benzothiazole;} \\
5-[^{123}\text{I}]-\text{i} & \text{odo-2-(4'}\text{'-trifluoromethylamido-3'}\text{'-methylphenyl)benzothiazole;} \\
5-[^{18}\text{F}]-\text{fluro-2-(4'}\text{'-amino-3'}\text{'-methylphenyl)benzothiazole; and} \\
5-[^{18}\text{F}]-\text{fluro-2-(4'}\text{'-trifluoromethylamido-3'}\text{'-methylphenyl)benzothiazole.}
\end{align*}
\]

5. A method for the *in vivo* diagnosis or imaging of amyloid-associated disease in a subject comprising administration of a compound of formula (I) or a salt thereof as defined in any of claims 1 to 4.

6. A radiopharmaceutical formulation which comprises a compound of formula (I) or a salt thereof as defined in any one of claims 1 to 4.
7. A compound of formula (Ia):

\[
\begin{array}{c}
\text{R}^1 \\
\text{N} \\
\text{H} \\
\text{R}^3 \\
\text{R}^2
\end{array}
\]

(la)

or a salt thereof, wherein:

\( R^1 \) is \(^{125}\text{I}, \ 1^{24}\text{I}, \ 1^{23}\text{I}, \ 75\text{Br}, \) or \(^{76}\text{Br}; \)

\( R^2 \) is C\(_{1-6}\) alkyl; and

\( R^3 \) is selected from hydrogen, C\(_{1-6}\) alkyl, -C(O) C\(_{1-6}\) alkyl, and -C(O)C\(_{1-6}\) haloalkyl.

8. A compound of formula (Ia) according to claim 7 wherein:

\( R^1 \) is \(^{125}\text{I}, \ 1^{24}\text{I}, \) or \(^{123}\text{I}; \)

\( R^2 \) is methyl; and

\( R^3 \) is selected from hydrogen and -C(O)C\(_{1-6}\) haloalkyl.

9. A compound of formula (Ia) according to claim 7 or 8 wherein the compound of formula (I) is selected from:

5-[\(^{125}\text{I}-\text{iodo-2-(4'-amino-3'-methylphenyl)benzothiazole};\]

5-[\(^{124}\text{I}-\text{iodo-2-(4'-amino-3'-methylphenyl)benzothiazole};\]

5-[\(^{123}\text{I}-\text{iodo-2-(4'-amino-3'-methylphenyl)benzothiazole};\]

5-[\(^{125}\text{I}-\text{ido-2-(4'-trifluoromethylamido-3'-methylphenyl)benzothiazole};\]

5-[\(^{124}\text{I}-\text{iodo-2-(4'-trifluoromethylamido-3'-methylphenyl)benzothiazole};\)and

5-[\(^{123}\text{I}-\text{iodo-2-(4'-trifluoromethylamido-3'-methylphenyl)benzothiazole}.\]
Figure 1 Percentage injected dose in brain

%ID Brain uptake of Compound 1

% Injected Dose

Time (mins)
Figure 2  Relative retention data

**Relative retention of Compound 1 in brain tissue**

<table>
<thead>
<tr>
<th>Time</th>
<th>5min</th>
<th>15min</th>
<th>30min</th>
<th>60min</th>
<th>120min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>![Bar Graph](Bar Graph_1.png)</td>
<td>![Bar Graph](Bar Graph_2.png)</td>
<td>![Bar Graph](Bar Graph_3.png)</td>
<td>![Bar Graph](Bar Graph_4.png)</td>
<td>![Bar Graph](Bar Graph_5.png)</td>
</tr>
</tbody>
</table>

Figure 3  Brain: blood ratio

**Brain:Blood tissue ratios of Compound 1**

<table>
<thead>
<tr>
<th>Time</th>
<th>5min</th>
<th>15min</th>
<th>30min</th>
<th>60min</th>
<th>120min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>![Bar Graph](Bar Graph_1.png)</td>
<td>![Bar Graph](Bar Graph_2.png)</td>
<td>![Bar Graph](Bar Graph_3.png)</td>
<td>![Bar Graph](Bar Graph_4.png)</td>
<td>![Bar Graph](Bar Graph_5.png)</td>
</tr>
</tbody>
</table>
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K51/04 C07D277/66

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

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07D

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

Electronic database consulted during the international search (name of database and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

Further documents are listed in the continuation of box C. Patent family members are listed in annex.

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

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

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

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone or in combination with another document or with common general knowledge

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

"A" document member of the same patent family

Date of the actual completion of the international search 14 May 2003

Date of mailing of the international search report 23/05/2003

Name and mailing address of the ISA
European Patent Office, P.B. 5816 Patentlaan 2 NL – 2280 HV Rijswijk Tel: (+31-70) 340-2040, Tx: 31 651 epo.nl, Fac: (+31-70) 340-3016

Authorized officer
Veronese, A

Form PCT/SA/210 (second sheet) (July 1992)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P,X</td>
<td>WO 02 085903 A (KUNG HANK ;KUNG MEI-PING (US); ZHUANG ZHI-PING (US)) 31 October 2002 (2002-10-31) page 6, paragraph 1 example 6 Fig.1 A, compound TZDM page 5, paragraphs 13-17 page 11, paragraphs 38,39</td>
<td>1-7</td>
</tr>
<tr>
<td>A</td>
<td>WO 01 14354 A (HUTCHINSON IAN PAUL ;POOLE TRACEY DAWN (GB); WESTWELL ANDREW DAVID) 1 March 2001 (2001-03-01) example 45</td>
<td>1-7</td>
</tr>
<tr>
<td>A</td>
<td>WO 97 26919 A (WARNER LAMBERT CO ;CAPRATH BRADLEY WILLIAM (US); GILMORE JOHN LOD) 31 July 1997 (1997-07-31) the whole document</td>
<td>1-7</td>
</tr>
</tbody>
</table>
INTERNATIONAL SEARCH REPORT

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 
   because they relate to subject matter not required to be searched by this Authority, namely:
   Although claim 5 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.: 
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.: 
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

Form PCT/SA/210 (continuation of first sheet (1)) (July 1996)
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>US 2003059369 A1</td>
<td>27-03-2003</td>
</tr>
<tr>
<td>WO 0114354 A</td>
<td>01-03-2001</td>
<td>AU 6708500 A</td>
<td>19-03-2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2382406 A1</td>
<td>01-03-2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 0114354 A1</td>
<td>01-03-2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2003507462 T</td>
<td>25-02-2003</td>
</tr>
<tr>
<td>WO 9726919 A</td>
<td>31-07-1997</td>
<td>AU 1529297 A</td>
<td>20-08-1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 9726919 A2</td>
<td>31-07-1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 6001331 A</td>
<td>14-12-1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZA 9700971 A</td>
<td>30-07-1997</td>
</tr>
</tbody>
</table>