Review Article

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# The development of PET radioligands for imaging the translocator protein (18 kDa): What have we learned?

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**The translocator protein (TSPO; 18 kDa), formerly known as the peripheral benzodiazepine receptor (PBR), is minimally expressed in the healthy brain. On the other hand, increased levels of TSPO have been noted in brain disorders for which an immune response is elicited. This increase in TSPO expression has been reported to coincide with the process of microglial activation making the measurement of TSPO density a useful indicator of active brain disease. To this end several new classes of TSPO positron emission tomography radioligands have been developed and evaluated. However, the incomplete pharmacological characterization of the TSPO and its ligands as well as differences in pathophysiology, pharmacology and molecular nature across species and tissue types means that caution must be exercised when comparing data obtained with various TSPO radioligands. A re-evaluation of our interpretation of imaging data, which better correlates with our current understanding of TSPO pharmacology in disease, requires consideration.**

**Keywords:** translocator protein (TSPO); PBR; PET; Carbon-11; Fluorine-18

## Introduction

The translocator protein (TSPO; 18 kDa), formerly the peripheral benzodiazepine receptor (PBR), is pharmacologically distinct from the central benzodiazepine-binding site on γ-aminobutyric acid-A ($GABA_A$) receptors.[1] It was discovered in 1977 when it was noted that the benzodiazepine, diazepam (**1**), bound to the mitochondrial fractions of rat kidney homogenate.[2] The TSPO is a highly hydrophobic, 18 kDa, tryptophan-rich protein with five trans-membrane spanning domains principally consisting of large α-helicies.[3,4] In the mitochondrial membrane, the 18-kDa TSPO associates with a 32-kDa voltage-dependent anion channel (VDAC) and a 30-kDa adenine nucleotide carrier (ANC), which forms part of the mitochondrial permeability transition pore.[4,5] This implicates the TSPO in the translocation of cholesterol from the cytosol to the inner mitochondrial membrane for conversion to pregnenolone by CYP11A1.[6] As cholesterol translocation is the first and rate-limiting step in all steroid biosynthesis, it is understood that the TSPO plays a crucial role in the modulation of neurosteroids.[7]

4′-Chlorodiazepam, Ro 5-4864 (**2**), was the first ligand to display high affinity for the TSPO but not for $GABA_A$ sites.[8] This was followed closely by the first non-benzodiazepine TSPO ligand, the isoquinoline carboxamide PK 11195 (**3**),[9] currently the most widely used compound for TSPO studies. Site-directed mutagenesis studies have demonstrated that the binding of **2** can be selectively removed without affecting the binding of **3**[10] to the TSPO, suggesting the existence of specific and separate benzodiazepine- and isoquinoline-binding sites.[11] The 18-kDa subunit has been selectively labelled with the isoquinoline carboxamide [$^3$H]PK 14105 indicating that this is the location of the isoquinoline-binding site, whereas the 30-kDa ANC and the 32-kDa VDAC have been selectively labelled with the benzodiazepines, [$^3$H]flunitrazepam and [$^3$H]AHN-086, identifying these two subunits as the location of the benzodiazepine-binding site. Although compounds in the benzodiazepine and isoquinoline classes bind to different sites on the TSPO, they appear to interact allosterically as binding has been shown to be mutually competitive at lower concentrations.[12]

Scatchard analysis, together with transmission electron and atomic force microscopy, has, in the presence of steroid-inducing hormones, revealed the existence of a second higher-affinity benzodiazepine-binding site.[13] In 1998, Li and Papadopoulos discovered, using further mutagenesis studies, a third site which allows for the binding of cholesterol **4**.[14] Given the large structural diversity across the known TSPO ligands and the existence of multiple binding sites on the TSPO, it is quite possible that not all of the ligands and radioligands evaluated to date bind to a common site and thus comparisons using the isoquinoline-binding site alone may produce erroneous results.

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Moreover, while the TSPO is abundantly expressed in the peripheral organs of healthy animals, the expression in the brain is negligible. Bacterial studies have also shown that the TSPO may form a functional dimer that constitutes the active state of the protein.[15] Significantly, porphyrins have been shown to bind with high affinity to the TSPO,[16] their internal plane of symmetry suggesting that they may bind to identical sites on the two components of a dimeric structure.[6,15] Thus, microglial activation and TSPO dimerization may contain additional binding sites not accessible in studies conducted using healthy animals, or indeed in *in vitro* studies, using monomeric TSPO. Recently, Sakai *et al*. demonstrated that [$^3$H]**3** retained its ability to bind to TSPO in Ehrlich tumour cells despite the absence of a single 18-kDa subunit. Interestingly the estimated $K_d$ of [$^3$H]**3** in Ehrlich tumour cells was 0.44 nM and 8.70 nM indicating two independent TSPO-binding sites. Furthermore, immunoblot analysis of mice adrenal, testis and Ehrlich tumour cells revealed the presence of TSPO dimers and trimers in the tumour cell lines.[17] Polymeric forms of TSPO may also be the predominant type in CNS disease states and thus the characterization of TSPO ligand binding and functional activity may be more effectively conducted using TSPO derived from activated microglia.

The anatomical distribution of the TSPO is largely restricted to the steroidogenic tissues of the periphery[18] and in some blood components.[19] In the CNS however, TSPO expression is almost exclusively limited to microglia, the brain's resident immune cells. While expression in the healthy brain is low, a marked upregulation is seen in active disease states, a process which has been shown to correlate with reactive gliosis and microglial activation.[20,21] This upregulation is seen in several neurodegenerative diseases, including Alzheimer's disease (AD),[22] Huntington's disease,[23] Parkinson's disease (PD),[24] ischaemic stroke,[25] and multiple sclerosis,[26] making the TSPO an attractive diagnostic and therapeutic target for the treatment and study of neurodegenerative diseases (Figure 1).[27]

## Imaging the TSPO

As a consequence of the marked upregulation of the TSPO in active disease states it has proven an attractive target for *in vivo* imaging of disease progression using functional imaging modalities such as positron emission tomography (PET).[28] This is particularly significant because this upregulation is seen to correlate with microglial activation and localized neuronal damage.[29]

### Benzodiazepines

In 1989, Junck *et al*.[30] attempted to use [$^{11}$C]**2** to image increased TSPO expression in human gliomas. Although **2** has been shown to have a high affinity for TSPO derived from rat kidney, [$^{11}$C]**2**, unlike the non-benzodiazepine [$^{11}$C]**3**, failed to show any accumulation in affected areas. Thus, while **3** maintains its affinity for the TSPO across species,[5] **2** does not.[31] This species difference has also been noted in cloning studies in which **2** displayed high affinity for rodent-derived TSPO ($K_D$ = 1–9 nM) but a much lower affinity for human TSPO ($K_D$ = 54 nM).[32] These findings, combined with results from mutagenesis studies, indicate the clear existence of separate isoquinoline- and benzodiazepine-binding sites.

### Isoquinoline Carboxamides

The first of the isoquinoline carboxamides to be radiolabelled was [$^{11}$C]**3** in 1984[33] and since then it has found favour in a number of animal and clinical studies. However, **3** has been shown to have a low uptake and specific binding in the healthy rat brain, while an increased uptake was noted in a striatal lesioned model.[34] It has also been shown that while enantiomerically pure [$^{11}$C]**3** shows no benefit over racemic **3** in healthy rats, the (*R*)-enantiomer shows a higher uptake in lesioned rat models.[35]

In 1992, Ramsay *et al*.[36] noted a marked upregulation of TSPO in a 55-year-old stroke patient using PET and [$^{11}$C]**3**. Quantitative PET studies using [$^{11}$C](*R*)-**3** in eight patients suffering from AD showed an increased regional uptake in the entorhinal, temporoparietal and cingulate cortices when compared with normal control who only showed an age-dependent increase in the thalamus. The correlation between the degree of uptake and disease progression indicated that microglial activation, as characterized by an increase in TSPO binding, plays an early role in Alzheimer's pathogenesis.[37] Similarly, it was noted using [$^{123}$I]iodo-**3** in SPECT imaging that patients with AD showed a mean increase in TSPO binding in neocortical regions compared with control subjects.[38] Indeed, increased [$^{11}$C](*R*)-**3** binding is seen in many forms of dementia with the highest upregulation observed in the frototemporal regions of the brain.[39] The TSPO also serves as a valuable marker of the gliosis seen in PD. PET imaging of [$^{11}$C](*R*)-**3** in 18 patients with clinically diagnosed PD revealed increased uptake in the pons, basal ganglia and frontal and temporal cortical regions when compared with normal controls.[24]

Despite these studies, a number of problems become evident with the use of **3** as a radioligand. First, the kinetic aspects of **3** greatly limit its clinical use as it has poor brain uptake and it binds extensively to plasma proteins.[40] A number of other isoquinoline derivatives have also been labelled with carbon-11 and fluorine-18, with no significant improvement on the kinetic properties of **3**.[41]

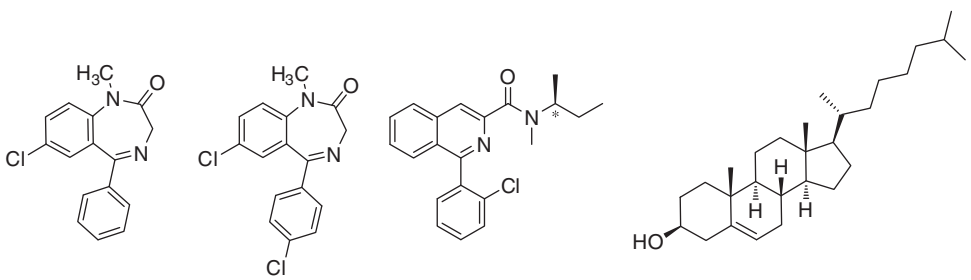



**Figure 1.** Diazepam (**1**) and TSPO-selective compounds, Ro 5-4864 (**2**), (*R*)-PK 11195 (**3**) and cholesterol (**4**).

## The need for new imaging agents

Owing to the poor performance of the benzodiazepines as imaging agents and the pharmacokinetic limitations of the isoquinolines, there has emerged a need for new imaging agents for the *in vivo* study of the TSPO.[42] Current evaluation of potential TSPO ligands mainly involves measuring the ability of a compound to displace [$^3$H]**3** from TSPO derived from rat kidneys,[43–45] which has resulted in numerous series of high affinity compounds.[46]

However, as it has been demonstrated that there are additional binding sites on the TSPO that are not related to isoquinoline compounds,[11,12,29] the current screening method falls short of addressing all potential binding sites. Furthermore, noted species differences suggest that *in vitro* results using animal-derived TSPO, as well as *in vivo* imaging of animals, cannot necessarily be translated to human studies.[30] Also, while establishing the $K_i$ of a compound allows for relative comparison of affinity it does not address the capacity to measure increased binding site density ($B_{max}$). Differences in $B_{max}$ and $K_D$ for TSPO ligands in some cases have better characterized their binding sites.[47] Finally, an increase in the number of binding sites on the TSPO as the result of steroid-inducing hormones[13] and the generation of additional binding sites through polymerization[6,15] may allow for the binding of TSPO ligands that were previously ignored because of their low affinity for inactive TSPO or microglia. A number of new classes of compounds have been developed as potential radioligands for imaging the TSPO and these are discussed in detail in this text and have been summarized in Table 1.

### The pyrazolopyrimidines

In 2001, the pyrazolopyrimidines were identified as a class of high affinity, selective TSPO ligands that are bioisosteres of the imidazopyridine, alpidem (**5**).[43] In 2005, Kassiou *et al.*[48] reported the radiolabelling and imaging of [$^{11}$C]DPA-713 (**6**) and a similar study was performed in 2008 using [$^{18}$F]DPA-714 (**7**)[50] in a healthy baboon. Both studies used an *in vitro* evaluation of the compounds in comparison with [$^3$H]**3**. In the case of [$^{18}$F]**7**, specific binding was demonstrated by pre-treatment with **3** in a quinolinic acid lesion in rats.

In 2006, Thominiaux *et al.* reported an improved radiosynthesis of [$^{11}$C]**6** using [$^{11}$C]methyl triflate, which is more amenable to automation and particularly advantageous where multi-dose preparations are required.[72] In 2008, Damont *et al.*[73] reported an improved one-step radiosynthesis of [$^{18}$F]**7**.

Recently, [$^{11}$C]**6** has been used for *in vivo* PET imaging of healthy human brain. High uptake was observed in all parts of the brain and the dose-normalized uptake was three times higher than that of [$^{11}$C]**3** (Figure 2).[74]

Direct comparisons of **6** and **3** were drawn in an AMPA-lesioned rat model of neuroinflammation by using microPET imaging of [$^{11}$C]**6** or [$^{11}$C]**3** followed by correlating immunohistochemical staining of neurons and microglia and autoradiographic studies using [$^3$H]**3**.[49] The study concluded that the higher ratio of specific to non-specific binding observed with [$^{11}$C]**6** compared with [$^3$H]**3** was most likely due to the lower lipophilicity of **6** and higher specific binding.[48–50,72–74]

A direct comparison of [$^{18}$F]**7**, [$^{11}$C]**6** and [$^{11}$C]**3** was made in a unilateral, striatal AMPA-lesioned rat using microPET followed by autoradiography. In this animal model [$^{18}$F]**7** possessed the highest ratio of specific to non-specific binding when comparing uptake in the lesioned and non-lesioned striatum.[51]

Doorduin *et al.*[75] evaluated [$^{18}$F]**7**, [$^{11}$C]**6** and [$^{11}$C]**3** in a rat model of herpes encephalitis using small animal PET. The authors found that uptake of [$^{11}$C]**6** in infected brain areas was comparable to that of [$^{11}$C]**3** but with the advantage of lower non-specific binding, making it more suitable for the detection of mild neuroinflammation. [$^{18}$F]**7** displayed low non-specific binding but the specific uptake was lower than that observed for [$^{11}$C]**6** and comparable to that observed for [$^{11}$C]**3**. This result is in contrast to that obtained from the previous striatum lesion model[51] and may be due to differences in the affinity state of the receptor between both animal models and the fact that [$^{18}$F]**7** is a TSPO agonist.

Martín *et al.*[76] evaluated [$^{18}$F]**7** in a rat model of focal cerebral ischaemia using *in vivo* PET imaging and, during the course of several days, observed a significant increase in radioligand uptake on the injured side compared with that in the contralateral area.

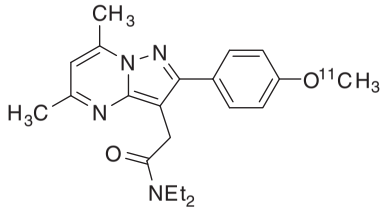
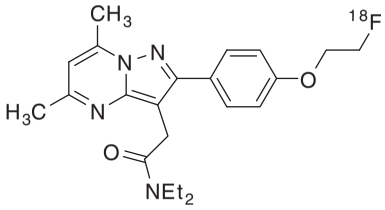
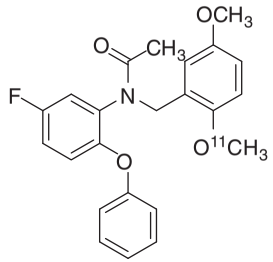
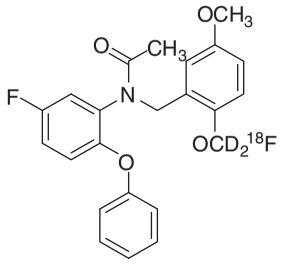
### Acetamides

The phenoxyphenyl acetamide [$^3$H]DAA1106 (**8**) was derived following structure–activity optimization of the ring opened analogue of **2**.[77] It was initially evaluated by an *in vivo* receptor labelling followed by an *ex vivo* microautoradiography by inhibition of [$^3$H]**3**[78] followed by binding to microglia derived from rat and monkey brain with non-specific binding determined using **8**.[77] This was followed by *in vivo* imaging of [$^{11}$C]**8** in healthy mouse brain[79] and kainic acid-lesioned rats[52] with specific binding demonstrated by blockade with **3** in both studies. The fluorinated ethyl analogue [$^{18}$F]FEDAA1106 (**9**) was imaged successfully in healthy rhesus monkeys[80] and a deuterated fluoroalkyl derivative [$^{18}$F]$d_2$FMDAA1106 (**10**) was also successfully imaged in a healthy monkey brain. The deuterated analogue reduced the previously seen *in vivo* defluorination of the non-deuterated analogue in mouse biodistribution studies.

In 2006, Fujimura *et al.*[56] used quantitative PET to evaluate [$^{18}$F]**9** in the living human brain. It was shown that [$^{18}$F]**9** had a six-fold higher signal compared with [$^{11}$C]**3** in seven healthy males (Figure 3).

In 2006, Zhang *et al.*[81] radiolabelled [$^{11}$C]DAA1097 (**11**), which showed good permeation of the blood–brain barrier in rat and accumulation in the occipital cortex of a healthy monkey. Both **8** and **11** were evaluated using crude mitochondrial preparations from rat brain, inhibiting both [$^3$H]**3** and [$^3$H]**2** binding with nanomolar or lower $IC_{50}$ values.[58] Competition studies showed that while ligands **8** and **11** displaced [$^3$H]**3** at nanomolar and picomolar concentrations, respectively, **3** was only able to displace [$^3$H]**8** at millimolar concentrations. This suggests that while both ligands **8** and **11** bind to a site, which shares similarities with the isoquinoline-binding site, they do not compete for this site alone. Furthermore, as recombinant TSPO was used it suggests that the additional binding displayed by **8** may occur on the TSPO itself. In a functional assay of steroidogenesis using MA-10 Leydig tumour and C6-2B glioma cells, DAA1097 (**11**) was shown to have similar functional properties to **3** (i.e. both are partial agonists in a steroid biosynthesis assay), whereas **8** did not elicit a response.[82] Quantitative autoradiography in rat brain also showed that DAA1097 (**11**) has a lower $K_i$ value when assayed against [$^{11}$C]**3**

**Table 1.** Current progress in TSPO radioligands

| Class | Compound | | Ref/s | | Suitability as a radioligand |
|---|---|---|---|---|---|
| Pyrazolopyrimidines | [$^{11}$C]DPA-713 (**6**) |  | 48,49 | Study performed in a healthy baboon. Specific and reversible binding demonstrated. MicroPET and immunohistochemical studies in an AMPA-lesioned rat model demonstrated that **6** provided a higher signal-to-noise ratio than **3** | Suitable binding and pharmacokinetics; however as an $^{11}$C ligand allows only for shorter imaging times |
| | [$^{18}$F]DPA-714 (**7**) |  | 50,51 | Evaluated in a healthy baboon and quinolinic acid-lesioned rat with specific and reversible binding successfully demonstrated. Direct comparison of **6** to **3** in an AMPA-lesioned rat model showed better signal-to-noise ratio | Slightly higher lipophilicity than DPA-713, but a stable $^{18}$F ligand allowing for longer imaging protocols |
| Phenoxyphenyl acetamides | [$^{11}$C]DAA1106 (**8**) |  | 52–54 | Imaged in healthy mouse brain and in kainic acid-lesioned rats, specific binding was demonstrated and expected increased activity was noted in lesioned animals | High specificity but shorter half-life limiting the complexity of imaging protocols |
| | [$^{18}$F]$d_2$FMDAA1106 (**10**) |  | 55 | Deuterated form of the fluoromethyl derivative of **8** was imaged successfully in a healthy monkey brain after the non-deuterated form defluorinated rapidly *in vivo*, resulting in bone uptake in mouse biodistribution studies | Pharmacokinetically; Deuteration does not prevent defluorination but rather slowed the process in mice resulting in bone uptake of [$^{18}$F]fluoride which may confound imaging studies |

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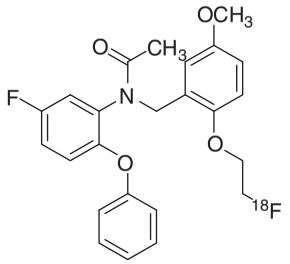
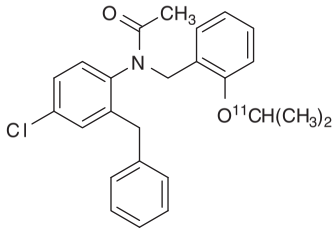
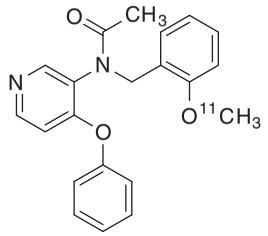
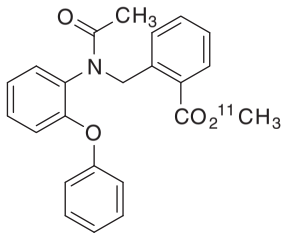
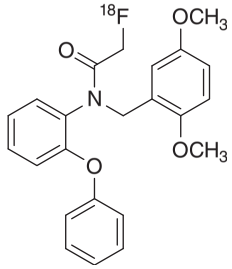
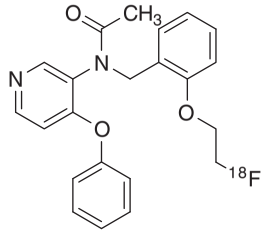
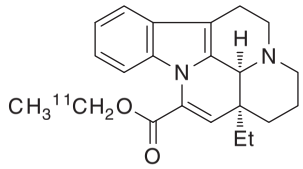
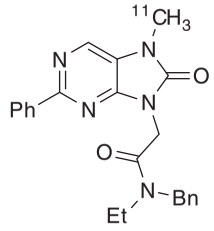
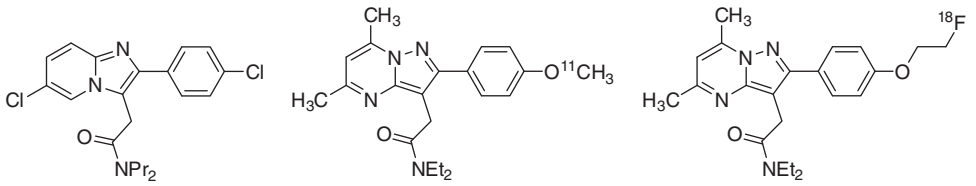
| | | | | |
|---|---|---|---|---|
| [$^{18}$F]FEDAA1106 (**9**) |  | 56,57 | Imaged successfully in a healthy rhesus monkey and evaluated in the human brain. [$^{18}$F]**9** had a six-fold higher signal-to-noise ratio than **3** and was stable to metabolism. Kinetics studies revealed metabolism occurred via debenzylation, resulting in a highly polar metabolite | Proven a more suitable imaging agent in humans with higher signal than **3** and a longer half-life allows for longer imaging times |
| [$^{11}$C]DAA1097 (**11**) |  | 58,59 | Showed very good permeation of the blood–brain barrier in rat and accumulation in occipital cortex of a healthy monkey | Limited number of studies to-date but good permeation of CNS warrants further investigation |
| [$^{11}$C]PBR28 (**12**) |  | 60–63 | Has been imaged in a healthy monkey showing specific binding and in a rat model of neuroinflammation localized uptake around areas of microglial activation noted. Biodistribution studies in both monkey and human showed a higher baseline level of activity in monkey with highest uptake in steroidogenic tissues of the periphery. | Suspected non-specific binding may potentially confound the validity of an imaging study |
| [$^{11}$C]PBR01 (**13**) |  | 64 | [$^{11}$C]**13** and [$^{18}$F]**14** were evaluated in monkey. Both compounds showed a high brain uptake. [$^{11}$C]PBR01 and [$^{18}$F]PBR06 both demonstrated specific and reversible binding. Although the kinetics of the two radioligands were similar [$^{18}$F]PBR06 performed better | *See below* |

**Table 1.** ***Continued***

| Class | Compound | | Ref/s | | Suitability as a radioligand |
|---|---|---|---|---|---|
| | [$^{18}$F]PBR06 (**14**) |  | | | Limited use in literature but slightly improved pharmacokinetics of **14** over **13** warrants its further use in imaging studies |
| | [$^{18}$F]FEPPA (**15**) |  | 65 | Imaging in rats showed high uptake in TSPO-rich regions, uptake and washout were slow and metabolism was rapid. Specific binding was not conclusively demonstrated | Specific binding needs to be established before use is increased |
| Vinca alkaloids | [$^{11}$C]vinpocetine (**16**) |  | 66,67 | [$^{11}$C]**16** successfully imaged in cynomolgous monkey, displacement of [$^{11}$C]**3** also demonstrated. Studies in young multiple sclerosis patients showed an increase in global uptake of [$^{11}$C]**3** and [$^{11}$C]**16**, however regional binding potential of [$^{11}$C]**16** was increased but not in [$^{11}$C]**3** suggesting different binding sites | Shows high potential as $^{11}$C radioligand, however binding site ambiguity needs to be resolved through *in vitro* studies to further validate studies, especially in models involving activated microglia |
| Aryl-oxodihydropurines | [$^{11}$C]AC-5216 (**17**) |  | 68–71 | Evaluated in murine fibrosarcoma model with heterogenous *in vivo* distribution around the edges of the tumour, specific binding demonstrated. In a kainic acid-lesioned rat model of neuroinflammation uptake was higher in affected areas and specific and reversible binding demonstrated. Studies in healthy animals have shown high distribution in steroidogenic tissues in mice and primates | Extensively and successfully used in both healthy animals and diseased states |

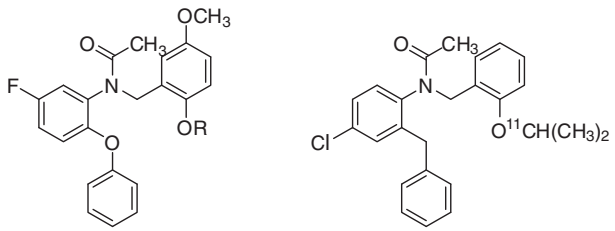


alipdem
**5**

[$^{11}$C]DPA-713
[$^{11}$C]**6**

[$^{18}$F]DPA-714
[$^{18}$F]**7**

**Figure 2.** The pyrazolopyrimidine bioisoteres of alipdem (**5**), [$^{11}$C]**6** and [$^{18}$F]**7**.



[$^{11}$C]DAA1106: [$^{11}$C]**8**, R = $^{11}CH_3$
[$^{18}$F]FEDAA1106: [$^{18}$F]**9**, R = $(CH_2)_2{}^{18}F$
[$^{18}$F]$d_2$FMDAA1106: [$^{18}$F]**10**, R = $CD_2{}^{18}F$

[$^{11}$C]DAA1097
[$^{11}$C]**11**

**Figure 3.** Phenoxyphenylacetamide series.

($K_i$ = 0.19 nM) compared with [$^{11}$C]**8** ($K_i$ = 0.68 nM), a trend which is reversed with **8** ($K_i$ = 0.95 nM for [$^{11}$C]**3**, $K_i$ = 0.16 nM for [$^{11}$C]**8**).[81]

In 2008, Venneti *et al.*[47] used an animal model of simian immunodeficiency virus encephalitis in macaques and patients with human immunodeficiency virus encephalitis to observe changes in the maximal binding site density ($B_{max}$) and dissociation constant ($K_D$) for [$^3$H]**8** and [$^3$H](*R*)-**3**. The authors demonstrated that the $B_{max}$ increased in encephalitic models of neuroinflammation, as expected, but more importantly the $B_{max}$ for **3** in both controls and diseased states were slightly different to that of **8**, indicating that **3** and **8** may bind to different sites. It has also been shown that **8** has a higher affinity for microglia/TSPO than **3** in rat models of neuroinflammation using lipopolysaccharide- and 6-hydroxydopamine-lesioned animals.[83]

In 2005, Briard *et al.*[60] reported the carbon-11 labelling of [$^{11}$C]PBR28 (**12**) (an analogue of DAA1106) which showed specific binding to TSPO in healthy monkey brain. In 2007, Imaizumi *et al.*[61] reported its use in a rat model of neuroinflammation using cerebral ischaemia showing that [$^{11}$C]**12** was a suitable marker of localized microglial activation. After administration to a rhesus monkey to evaluate biodistribution, [$^{11}$C]**12** was administered to seven healthy human subjects[62]; the monkey baseline levels of activity were higher than human. Generally, uptake was highest in steroidogenic tissues of the periphery and organs involved with metabolism and excretion. In addition, one unusual human subject showed reduced activity, which was inconsistent with expectation and apparently due to the absence of the appropriate binding protein. Kinetic studies of [$^{11}$C]**12** were carried out by Fujita *et al.*[63] in 2008 by using PET imaging in healthy humans combined with arterial sampling. It was found that the quantified level of TSPO in the human brain was approximately 5% of that in monkey. Interestingly, again 2 of the 12 human subjects appeared to have no binding protein present, no explanation is offered for this but with these exceptions it was concluded that [$^{11}$C]**12** is still a promising ligand for *in vivo* study of the TSPO. Imaizumi *et al.*[84] also evaluated [$^{11}$C]**12** as a PET imaging agent in healthy monkeys and found a high specific uptake in the brain with regional distribution proportional to the density of TSPO (Figure 4).

[$^{11}$C]PBR01 (**13**) and [$^{18}$F]PBR06 (**14**) were evaluated in monkey as potential imaging agents for human studies. Both compounds showed a high brain uptake, [$^{11}$C]**13** was selectively blocked using **13** and [$^{18}$F]**14** was blocked using **8**, resulting in rapid washout in each case. Although the kinetics of the two radioligands were similar, [$^{18}$F]**14** performed slightly better, most likely due to the longer half-life of fluorine-18 allowing for longer acquisition time.[64,85] Fujimura *et al.*[86] evaluated [$^{18}$F]**14** as a PET imaging agent in healthy human brain and concluded that it was a longer-lived and promising alternative to $^{11}$C-labelled radioligands for measuring neuroinflammation despite an accumulation of radiometabolites in the brain.

[$^{11}$C]**13**, [$^{11}$C]**12** and **8** were evaluated *in vitro* by displacement of [$^3$H]**3** from mitochondria derived from rat, monkey and human brain. Compared with **3**, all three compounds showed a higher affinity for the TSPO and a higher affinity for rat-derived TSPO compared with that derived from monkey mitochondrial fractions. For all three ligands the lowest affinities were observed for human-derived TSPO, with **8** showing the highest affinity. In general screening, at a concentration of 10 μM, all three compounds showed less than 50% displacement of reference compounds for seretonergic, GABAergic, adrenergic, histaminergic, muscarinic and dopaminergic receptors, indicating a high degree of selectivity.[87]

Using rat cortex-derived TSPO, [$^{18}$F]-FEPPA (**15**), the fluoroethoxy derivative of **12**, was evaluated *in vitro* and was found to displace [$^3$H]**3** with high affinity ($K_i$ = 0.07 nM). After intravenous injection into rats, slow brain uptake and slow washout was observed with the highest uptake in the TSPO-rich regions of the hypothalamus and olfactory bulb. Metabolism was rapid and attempts to determine the binding specificity were complicated by the absence of a reference region devoid of TSPO in the rat brain for measuring the non-specific binding. Similarly, pre-blocking studies also failed in the determination of specific binding due to a possible correlation between increased plasma levels of radioligand and the potential for brain uptake.[65]

## Vinca alkaloids

The vinca alkaloid, [$^{11}$C]vinpocetine (**16**), is the only semi-synthetic TSPO ligand that has thus far been used in *in vivo* imaging. Pre-treatment of two cynomolgous monkeys with vinpocetine reduced [$^{11}$C]**3** uptake, whereas pre-treatment with **3** increased [$^{11}$C]vinpocetine uptake (by peripheral blockade of TSPO) but reduced the binding potential of [$^{11}$C]vinpocetine.[88]

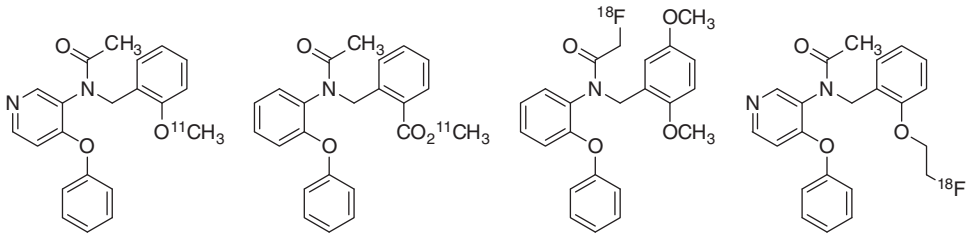



**Figure 4.** PBR28 and similar derived compounds.

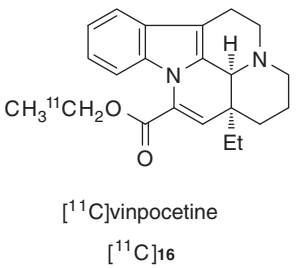



**Figure 5.** [11C]vinpoecetine.

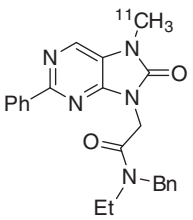



**Figure 6.** [11C]AC-5216.

These observations also led the authors to propose that the clinical neurological effects of vinpocetine may be due to its effect on microglial cells (Figure 5).

In 2008, Vas *et al.*[66] used PET imaging to show increased uptake of [11C]**3** and [11C]**16**, both globally and locally, in the CNS of four young multiple sclerosis patients. However, while [11C]**16** showed an increase in regional binding potential in affected areas of the CNS, **3** did not. This suggests that the two radioligands may bind to different sites on the TSPO or that their binding may depend on the active state of microglia.

### Aryl-oxodihydropurines

[11C]AC-5216 (**17**) is a high affinity, purine TSPO ligand. **17** displayed an ability to potently displace [3H]**3** from rat C6 and human Hs683 glioma-derived mitochondrial fractions as well as high binding affinity when tested in whole rat brain-derived TSPO (Figure 6).[68]

When [11C]**17** was injected into a murine fibrosarcoma model the *in vivo* distribution was heterogenous, showing a high accumulation around the edges of the tumour with little in the centre. Co-administration with **3** resulted in a homogenous distribution of activity suggesting specific binding. *In vitro* autoradiographic analysis showed some discrepancy with the homogeneity of [11C]**17** distribution, suggesting that the delivery of radioligand from the plasma to the tumour may be the rate-limiting step in the fibrosarcoma model.[69] In 2007, Yanamoto *et al.*[70] evaluated [11C]**17** in a kainic acid-lesioned rat model of neuroinflammation; lesioned animals showed an increase in [11C]**17** binding compared with non-lesioned animals, which was blocked by administration of **17** and **3**, demonstrating specific TSPO binding. Further studies have shown that [11C]**17** displays a high accumulation in TSPO-rich tissues in mice and primates, which can be blocked by treatment with **17** and **3** and metabolite studies have shown that while [11C]**17** is stable in the mouse brain it undergoes metabolism in the plasma of mice and monkeys.[71]

## Conclusion

Although the TSPO has been the focus of numerous studies for more than 30 years, our current understanding of its role in pathophysiology is still not completely understood. Efforts to elucidate the role of the TSPO in both health and disease have been hampered by a number of fundamental challenges unique to the TSPO as both a pharmacological and imaging target.

First, during the process of microglial activation a number of molecular changes occur that affect both the structure and function of the TSPO. These changes include an increase in binding site density, increased expression[4] and polymerization,[15] which may result in additional sites for binding compared with those found on resting microglia. To date, most imaging studies performed in the non-human primate brain have been conducted in healthy animals, thereby imposing limitations on our ability to critically assess the utility of a TSPO radioligand as a marker of neuroinflammation in the CNS. Furthermore, molecules may bind to additional TSPO sites with high affinity only when microglia are activated.[89] Many have concluded that high brain uptake in healthy animals is a reliable measure of a good TSPO radioligand.[90] This is in error due to the fundamental changes that occur in the TSPO during active disease states. High brain uptake does not always correlate with the ability to image activated microglia or increased expression of TSPO.

While [11C]**3** has a number of limitations, which preclude its use as an imaging agent in neuroinflammation, it is also not possible to consider new imaging agents as challengers to **3**[91] unless they are competitive binders. As a number of studies have demonstrated that multiple binding sites exist, it cannot be assumed that all new TSPO radioligands are only competitive

binders for the isoquinoline site.[10,11] Moreover, use of different animal models makes the comparison of radioligands difficult as the level of microglial activation and/or TSPO expression may depend on the model used. Additional binding sites may become accessible during microglial activation as a result of polymerization[6]. Further elucidation of these binding sites is necessary to better interpret the results of TSPO imaging studies.

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