[18F]T807: A Tau Selective Radioligand For PET Imaging Of Neurodegenerative Disease

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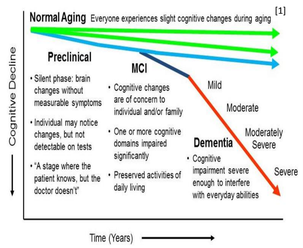
1. Neurodegenerative Disease – Alzheimers'

Alzheimer Disease (AD) is one of many progressive neurodegenerative diseases affecting more than 1/2 a million people in the UK alone today. Neurodegenerative diseases such as AD fall into the category of diseases known commonly as 'dementia' , a condition characterised by the atypical accumulation of proteins known as β-amyloid plaques, fibres called tau tangles and the neurotransmitter acetylcholine (AChE) in the brain .

This results in a gradual degradation and death of brain cells, atrophy which consequently gives rise to symptoms such as memory loss and a reduced ability of the brain to process information leading to issues with understanding, judgement, speech and language, among many other symptoms which are associated with a reduced mental capacity. However the trigger for the onset of this process is currently unknown.[1],[3]

[1]



[2]

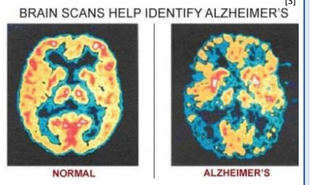
As neurodegenerative diseases are characterised by the microtubule protein tau, in the brain creating neurofibrillary tangles. The synthesis of a tau selective radioligand, like [18F]T807, for PET imaging of neurodegenerative disease is important to enable early detection and identification of a possible cause. This would improve the quality of life of those prone to such diseases and is therefore imperative .[4]-[7]

Hence this area of research is crucial to provide a greater understanding of these diseases; where there are currently a limited range of treatment options, with existing treatment being limited to drugs that delay the progression of the disease or invasive surgery to remove part of the brain. However these treatments fail to address the cause of the disease and can only be applied once the individual has been diagnosed which is usually too late hence a new solution is needed.

2. Introduction to PET Imaging

Positron-emission tomography (PET) is a functional nuclear medical imaging technique which utilises minute quantities of radioactive material in the form of radiotracers. Radiotracers possessing short half-lives are injected into the body intravenously, and through a series of steps, ultimately produce a final 3D-image of the body. This is important in aiding the diagnosis of diseases and to determine the stage and severity the patient is at. PET scans can be used for the investigation of various illnesses in the areas of oncology, neuroscience, cardiology, stem cells, diabetes etc. In comparison to CT or MRI scans, PET scans have the added advantage of being able to look at the function, as well as the structure and size of organs and tissue within the body, and are consequently used in combination with these scanners. [3]-[7]

[3]

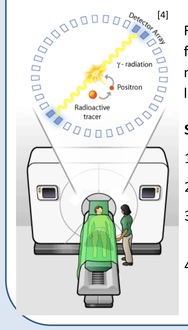


A brain scan done with PET illustrating a comparison between a normal brain and the brain of someone with Alzheimer's disease proving a visual discrepancy in the activity within the brain. Reduced activity in the brain as a result of Alzheimers disease is illustrated by the areas in black and blue.

Why is medical imaging useful ?

Medical imaging provides a deeper understanding and illustration of the inner workings of parts of the human body including the brain, and is becoming more and more valuable for both clinical use and drug development and diagnosis. In stark contrast to alternative diagnostic methods such as excision, tissue sampling or fluid analysis, such non-invasive imaging techniques provide a clear 3D visual image illustrating the whole organ at different time points whereas biopsies only take a single sample at a single time point of one part of the organ. [3]-[7]

[4]



Radioisotopes that are often used for PET imaging are carbon-11, oxygen-15 and most commonly fluorine-18 due to its advantageous properties such as having an optimal half-life of 110 min, good metabolic stability due to the strong C-F bond, and the ability to produce high resolution images with a low positron energy of 0.202 MeV. [3]-[7]

Steps involved in PET imaging :

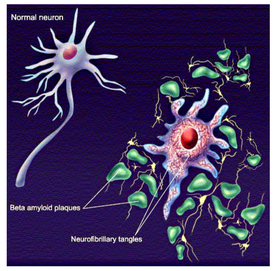
1. Prior to the PET scan the patient is injected with a radiotracer.
2. The radioactive tracer decays resulting in a positron being emitted.
3. Two gamma rays are generated, as the result of annihilation of the liberated positron with electrons, which then escape from the body.
4. The gamma rays, which are generated in opposite directions, are then detected using a PET scanner which is used in conjunction with a computer which then tracks the movement of the radioactive tracer creating a 3D image of the brain. [3]-[7]

3. β-Amyloid vs. Tau

β–Amyloid (Aβ) hypothesis

Patients with AD have been shown to possess abnormal accumulation of β-amyloid plaques in neurons within the brain. These insoluble plaques are derived from amyloid precursor protein (APP) which is a peptide component of the cell membranes of the neurons. Enzymes can cut APP resulting in peptides being released into the extracellular space of the neuron (outside the cell) which can then aggregate creating oligomers (polymer consisting of monomers) which interfere with the normal function of the neurons. These build up and are deposited in the brain, clumping together to create β-amyloid plaques. Neuroimaging techniques for these plaques, with radiotracers such as [11C]PIB and [18F]AV-45 ([18F]Florbetapir), can be used to aid diagnosis of AD. [5]-[9]

[9]



Tau hypothesis

Tau (Tubulin-associated unit) is a phosphorylated protein that forms around nerve endings in a intracellular fashion, compared to β-amyloid plaques which accumulate extracellular. Tau plays a key role in AD as it is important in binding to axonal microtubules in neurons and stabilizing them allowing the control of substances within cells and effective biochemical communication between neurons. The degree of binding of tau to these microtubules is determined by the extent of phosphorylation (addition of phosphate PO_4^{3-} groups). Hyperphosphorylation of tau results in weaker binding resulting in dissociation of tau which is able to then manifest into large fibrils or aggregates called paired helical filaments (PHFs); these accumulate in neurons causing neurofibrillary tangles which are characteristic of AD. Therefore Tau PET imaging agents can be used to monitor the progressive dysfunction and death of neurons. The Table below illustrates some PET radiotracers, with [18F]T807 in humans being the most promising, demonstrating high selectivity (>25-fold) and affinity for tau despite having slower kinetics than β-amyloid tracers. [5]-[9]

Table 1: Leading tau selective radiotracers[5]

Origin	PET imaging agent	Affinity for Tau [nM]	Specificity for Tau compared to Aβ	Mouse brain uptake at 2 min[%ID/g]	Mouse brain washout (2 min/30 min)
Lilly	[18F]T807	K_d= 14.6*	>25-fold	4.16	6.7
TOHOKU UNIVERSITY	11C-PBB3	K_{d1} = 2.5* K_{d2}= 100	40-50-fold	NA	NA
TOHOKU UNIVERSITY	18F-THK5117	(K_1= 10.5)≠	NA	6.06	10.3

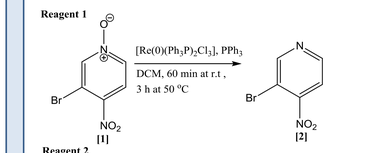
*K_d regulated by the binding of the ligand to sections of the brain which are tau-positive

NA = not available

4. Aims and Outcomes

- To identify routes to 3-bromo-4-nitropyridine **[2]** from 3-bromo-4-nitropyridine-*N*-oxide **[1]**
- To prepare gram quantities of 3-bromo-4-nitropyridine **[2]** using an optimised process
- To investigate the synthesis of the intermediate nitrobiaryl **[4]** using a Suzuki reaction
- To identify a method for the synthesis of carbazole **[5]**
- To prepare and isolate carbazole **[5]**
- To investigate the synthesis of [19F]T807, from **[5]**, using a second Suzuki reaction
- To determine the absorbance and emission spectra for [19F]T807
- To provide material for a range of bioassays

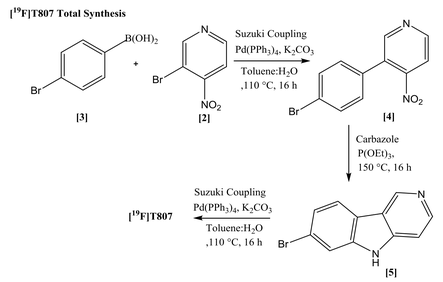
5. Synthesis of [19F]T807



Preparation of reagents

A suitable route for the production of 3-bromo-4-nitropyridine **[2]** from its precursor 3-bromo-4-nitropyridine-*N*-oxide **[1]** was needed. This was achieved using a rhenium catalyst $[Re(0)(Ph_3P)_2Cl_3]$ and triphenylphosphine in dichloromethane under a nitrogen atmosphere, providing the target arene **[2]** in a yield of 95% (Reagent 1)

The commercial material for **[3]** failed to generate the nitrobiaryl **[4]** in the palladium catalysed Suzuki reaction. This was attributed to dehydration of the material giving the boroxine **[3a]** which was then converted to **[3]** in quantitative yield by heating in water (Reagent 2).



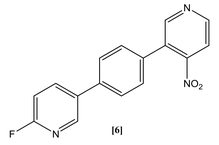
Construction of the aromatic core of [19F]T807

With the two reagents now available the focus moved to the formation of the key aromatic framework of [19F]T807 (Total Synthesis).

The first step was formation of nitrobiaryl **[4]** which was achieved in good yield (91%) using a Suzuki reaction (step 1). Conversion of this nitrobiaryl species to carbazole **[5]** was achieved using a reductive cyclisation protocol, catalysed by $P(OEt)_3$, generating the product as a bright orange solid (76%, step 2).

The final step to produce [19F]T807 was then carried out using a second Suzuki reaction‡ (step 3).

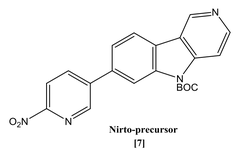
6. Future Work



Parallel studies:

Ongoing studies have considered the order of transformation detailed above (see Total Synthesis).
The reaction conditions to prepare nitrobiaryl **[4]** were optimised using microwave heating as this proved to be more time effective as well as improving the yield of the reaction (μW, 110 ° C , 120 W for 30 min).

It was found that the final two steps in the total synthesis to produce **[6]**, could be carried out in the opposite order. Triphenylphosphine (PPh_3) was then used as the Lewis acid and DMA as the solvent (at 150 ° C) to facilitate the purification of [19F]T807 by simplifying the removal of the phosphine derived by-products.



Future work:

- Determination of the selectivity for tau/potency of [19F]T807 using a range of bioassays
- Investigate the route to the nitro-derivative **[7]** as this material is needed as both the radiolabelling precursor and QC reference material
- Development of a validated QC method for [18F]T807 radiopharmaceutical production
- Preclinical production of [18F]T807 and associated imaging/auto-radiography
- Translation of [18F]T807 to the clinical setting

7. Conclusions

- Preparation of the key reagents, **[2]** and **[3]**, was achieved
- The nitrobiaryl intermediate **[4]** was produced in good yield using a Suzuki reaction
- The reductive cyclisation to give carbazole **[5]** was also successful
- This work provided insight into the properties of this class of compounds, the purification techniques used, and provided material for further studies (e.g. bioassays)
- Access to the 'cold' standard [19F]T807 is essential in the development of the tau selective radioligand [18F]T807, which shows promise in the earlier and more precise diagnosis of AD and other neurodegenerative diseases using PET imaging

7. References

[1] Will quantum physics help us cure Alzheimer's disease?,http://wavefunction.fieldofscience.com/2012/01/will-quantum-physics-help-us-cure.html,(Date accessed 20/10/2014). [2] What is Alzheimer's Disease? , http://www.alzheimers.org.uk/site/scripts/documents_info.php?documentID=100,(Date accessed 18/10/2014). [3] Alzheimer's Disease: a 21st Century Epidemic, http://www.lmreview.com/articles/view/alzheimers-disease-a-21st-century-epidemic/,(Date accessed 20/10/2014). [4] Looking inside the human body using positrons, http://www.cidpusa.org/PET_Scan.html, ,(Date accessed 30/10/2014). [5] M.Shah, A.M.Catafau, *J. Nucl. Med.*, 2014, **55,** 871-874. [6] V.L.Villemange, N.Okamura, *Alzheimer's & Dementia*, 2014, **10**, 254-264. [7] S.Shokouhi, D.Claassen, W.R.Riddle, *J Alzheimers Dis Parkinsonism*, 2014, **4**, 1-4. [8] A.Mudher, S.Lovestone, *Trends in neurosciences*, 2002, **25**, 22-26. [9] Pathophysiology, https://sites.google.com/site/bme365ralzheimers/pathophysiology, (Date accessed 18/10/2014)

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