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Metal complexes designed to bind to amyloid-β for the diagnosis and treatment of Alzheimer’s disease

David J. Hayne, SinChun Lim and Paul Donnelly

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Alzheimer's disease is the most common form of age-related neurodegenerative dementia. The disease is characterised by the presence of plaques in the cerebral cortex. The major constituent of these plaques is aggregated amyloid-β peptide. This review focuses on the molecular aspects of metal complexes designed to bind to amyloid-β. The development of radioactive metal-based complexes of copper and technetium designed as diagnostic imaging agents to detect amyloid burden in the brain is discussed. Separate sections of the review discuss the use of luminescent metal complexes to act as non-conventional probes of amyloid formation and recent research into the use of metal complexes as inhibitors of amyloid formation and toxicity.

1 Brief introduction to the pathology of Alzheimer’s disease

Alzheimer’s disease (AD) is a progressive neurodegenerative condition that results in synaptic failure and neuronal death. These symptoms initially manifest as mild forgetfulness but lead to complete loss of cognition. Characteristic pathological hallmarks in the brains of those suffering with the disease include the presence of extracellular senile plaques, intracellular neurofibrillary tangles and altered levels of neurotransmitters. Amyloid plaques are composed of an insoluble aggregated peptide called amyloid-β (Aβ). This peptide is 39–43 residues in length and derived from the amyloid-β precursor protein (APP). The amyloid plaque burden in subjects does not consistently correlate with cognitive impairment and some argue that smaller soluble oligomeric species are the toxic species responsible for neuronal death. However, oligomers and plaques are thought to be in equilibrium. Although the exact role of amyloid plaques in the onset of dementia is controversial, what is certain is that histopathological studies show extensive cortical Aβ deposition in post-mortem analysis of AD subjects. Neurofibrillary Tangles (NFT) consist of a hyper phosphorylated form of a microtubule-associated protein called tau. NFT initiate with the formation of bundles of paired helical filaments that accumulate in the neuronal cytoplasm. The hyper-phosphorylation of tau results in its detachment from microtubules that consequently lose structural integrity with concomitant impaired axonal transport and compromised synaptic function. Another prominent and consistent feature of AD is deficits to the acetylcholine (cholinergic) neurotransmitter system. Post-mortem studies have revealed cholinergic neuronal loss and reduced acetylcholine levels. This correlates with a decrease in the concentration of acetylcholine esterase, an enzyme responsible for the hydrolysis of acetylcholine in the brain.

The focus of this review is metal complexes designed to bind to either Aβ fibrils or Aβ plaques as distinct from metal-binding "free" ligands designed to displace either copper or zinc or iron ions that are thought to be bound to Aβ plaques. The approaches that are discussed in this review include: the potential of radioactive copper and technetium complexes to be used as diagnostic imaging agents to elucidate plaque burden in living patients; the use of luminescent metal complexes as non-conventional probes of amyloid formation; and the use of platinum, ruthenium, iridium and rhodium metal complexes to inhibit the aggregation and toxicity of Aβ. Several elegant and sophisticated approaches that use chelating ligands designed to attenuate Cu(II)/Zn(II)-Aβ interactions and redistribute metal ions bound to Aβ plaques that are of significant interest as potential therapeutics will not be discussed in this review but selected leading references and reviews are available.

2 The amyloid-β peptide and plaques

The amyloid precursor protein (APP) is encoded by a single gene on chromosome 21 and resembles a cell-surface receptor having a single transmembrane domain, a large extracellular domain and a short cytoplasmic tail. The transmembrane domain extends into the intracellular region and contains the C terminus while the N terminus resides within the extracellular domain. Two pathways exist for the processing of APP, one being non-amyloidogenic whilst the other pathway is amyloidogenic (Figure 1). Non-amyloidogenic metabolism involves cleavage of APP by α-secretase, a membrane anchored secretase, releasing the soluble N terminus fragment, sAPPα, to the extracellular space. The remaining transmembrane fragment is cleaved by γ-secretase within the transmembrane domain liberating non-amyloidogenic...
P3 (Aβ17-40 or Aβ17-42) to the extracellular space. The amyloidogenic proteolysis of APP follows a similar series of events but is cleavage by β-secretase (also known as β-APP cleaving enzyme or BACE-1) followed by γ-secretase releases sAPPβ and Aβ. Aβ ranges in length from 39–43 amino acids depending where γ-secretase cleavage occurs giving variability at the hydrophobic C-termini connected to the hydrophilic N-terminal domain. The two main peptides produced are 40 (Aβ40) and 42 (Aβ42) amino acid residues in length; Aβ42 shows the greater propensity for aggregation in vivo and is often considered the more toxic. As monomers, the peptides show unfolded random coil conformation with some α-helical and β-sheet structure. An imbalance between the production and clearance of Aβ peptide results in its accumulation as oligomers, protofibrils, fibrils, and then extracellular Aβ plaque deposits in the brain parenchyma. The amyloid hypothesis argues that Aβ plaques and/or their precursors trigger a cascade of events leading to synaptic dysfunction, microgliosis, and neuronal loss. Aβ fibrils are not crystalline and generally insoluble in aqueous solvents. This precludes analysis by conventional solution NMR. Nevertheless, recent advances in cryoelectron microscopy and solid-state NMR have provided structural information on the inter- and intra-molecular interactions that bind the cross-β structural motifs of Aβ fibrils. Structural studies using fibrils formed from 35MoxAβ42 peptides (containing a methionine sulfoxide at position 35) have given insight into the 3D structure of Aβ fibrils (Figure 3). The mechanism is oxidised during the production of the monomeric peptide but maintains similar properties to the non-oxidised Aβ. A single 35MoxAβ42 protofilament consists of two β-sheets (β1 and β2) that run along the fibril axis forming a parallel β-sandwich stabilized by inter-β-sheet side-chain interactions. Residues 17-26 define sheet β1 and residues 31-42 form sheet β2 with a connecting loop containing residues 27-30. Intramolecular interactions between amino acid residues on β1- and β2-sheets involve ionic interactions (Asp23 and Lys28), hydrogen bonds (Leu17, Phe19, Ala21, Gly38 and Val36) and backbone hydrogen bonds. The loop region and β1 interact by salt bridge formation between the side-chains of residues Asp23 and Lys28. Residue Lys28 also forms close contacts with residues Ile32 and Leu34 of β2. The intermolecular hydrophobic interaction formed between the odd-numbered residues of the β1-strands (Leu17, Phe19 and Ala21) and the even-numbered residues (Gly38 and Val36) of neighbouring β2-strands establishes the hydrophobic core of Aβ fibrils. The extension and growth of the Aβ42 protofilament is unidirectional; an incoming n-th Aβ42 monomer can initiate contact with the fibrillar end by means of hydrophobic interactions between its β1-strand and the β2-strand of the (n-1)th monomer. The ionic interactions between the residues in the loop regions and backbone hydrogen bonds further strengthen the intermolecular association. In order to prevent the dissociation of the n-th monomer, another Aβ42 monomer is required to interact.

Figure 2. Schematic representation of amyloid fibril. (a) β-strands are arranged perpendicular to the fibril axis (axis shown in red, side-chains shown as spheres). (b) Two sheets are paired by the interlocking of side-chains, the two lighter coloured peptides in each sheet highlight this and correspond with the lighter coloured peptides in (a). (c) β-sheets twist together to form a protofibril which aggregates to form the mature fibril (d) (direction of fibril axis shown with red arrow). Reproduced with permission from Skeby, K. K.; Soerensen, J.; Schnietz, B. J. Am. Chem. Soc. 2013, 135, 15114. Copyright 2013 American Chemical Society.

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with the β2-strand of the nth monomer to stabilize it. This suggests that the sequence-selective and cooperative mechanism of Aβ fibril growth is a first-order kinetic process.56

Figure 3. (a and b) Ribbon diagrams of the protofibril core structure (residues 17–42) illustrating the intermolecular nature of the β-strand interactions. Aβ monomers are individually colored. The β-strands are indicated by arrows, non-regular secondary structure is indicated by spline curves through the Cα atom coordinates of the corresponding residues. The bonds of side chains that constitute the core of the protofilament are shown. (b) Dotted lines indicate ion pair interactions forming intermolecular salt bridges between residues D23 and K28, two salt bridges formed by the central Aβ42 molecule are highlighted by rectangles. (c) Van der Waals contact surface polarity with a ribbon diagram at the odd end of the 35Mox Aβ42 protofibril showing residues 17–42. The colours of side chains indicate if they are hydrophobic (yellow), polar (green), negatively charged (red), or positively charged (blue). Positively and negatively charged surface patches are shown in blue and red, respectively, and all others are shown in white. (d) (Upper) Simulation of a 35Mox Aβ42 fibril that consists of four protofilaments colored individually. Lower shows the same fibril in a noisy gray-scale image, which has been blurred corresponding to a resolution of 2 nm. In Right, a ×5 magnified cross section perpendicular to the fibril axis is shown, using the same color code. Dimensions are indicated. To match the experimental twist of the protofilament of the fibril shown in (e), a twist angle of 45° per molecule was used. (e) Two examples of cryoelectron micrographs of single 35Mox Aβ42 fibrils. The direction of the fibril axis is indicated by the direction of the black arrows (Scale bar, 50 nm). Adapted with permission from Luhrs, T.; Ritter, C.; Adrian, M.; Riek, Loher, D.; Bohrmann, B.; Dobeli, H.; Schubert, D.; Riek, R. Proc. Natl. Acad. Sci. U.S.A., 2005, 102, 17342. Copyright 2005 National Academy of Sciences, U.S.A.

3 Structural morphology of amyloid plaques

The relationship between soluble Aβ monomers, oligomers Aβ, protofibrils, fibrils and plaques is complicated and several aspects of the dynamics remain uncertain.55–58 Amyloid-β plaques are classified into two morphological types: (i) neuritic and (ii) diffuse (“pre-amyloid”) plaques (Figure 4). Neuritic plaques, observed after staining, have a dense compact spherical appearance with a diameter ranging from 10 μm to greater than 120 μm. Neuritic plaques can be further sub-classified into ‘fibrillar’ or ‘dense-cored’ plaque forms. Fibrillar plaques show dense accumulations of Aβ throughout the plaque structure (Figure 4B) whereas cored plaques have a distinct central core of Aβ encircled by a void or clearing surrounded by an outer spherical rim of Aβ (Figure 4A). These Aβ deposits are referred to as neuritic plaques as they are spatially localized with dystrophic neuritis (clusters of abnormal neuronal processes) both inside and immediately surrounding the deposits.57–59

Figure 4 Serial sections of human brain tissue displaying Aβ plaques of different morphologies. (A, i-iii) Optical sections of a dense core plaque, observed after staining with a histological dye for amyloid. Images were captured at ~14.5 μm intervals and show the dense Aβ core of the plaque (arrow) (A, ii) surrounded by a void defined by an outer rim of Aβ (arrow heads). (B, i-iii) Similar images of a fibrillar plaque with images captured at intervals of ~17.5 μm. Spoke-like Aβ accumulations (arrow) radiate from the dense central accumulation. (C, i-iii) Confocal images of a diffuse plaque after treatment with an Aβ antibody. The images were captured at intervals of ~2.5 μm and show the punctate accumulation of Aβ giving a granular pattern. Scale bar = 20 μm (A and B), 50 μm (C). Reproduced with permission from T. C. Dickson and J. C. Vickers, Neuroscience, 2001, 105, 99–107. Copyright 2001 Elsevier.

Following staining, diffuse plaques display a finely granular pattern of amorphous shape that lacks a fibrillar, compacted centre (Figure 4C). These non-fibrillar plaques are the only Aβ deposits found in regions of the brain not clearly associated with the typical symptoms of AD. They also form within the same regions of the brain as neuritic plaques, however, very little or no detectable dystrophic neurites are associated with diffuse plaques. Studies in a transgenic AD mouse model, expressing mutant human APP, show that the mice develop diffuse plaques before fibrillar plaques supporting the hypothesis that diffuse plaques are immature or precursor lesions to neuritic plaques and are therefore also termed “pre-amyloid” plaques.59

4 Molecules that bind to Aβ fibrils and plaques

The histopathological dyes Congo Red and Thioflavin T (ThT) (Figure 5) have provided the structural inspiration for the development of many radiolabeled tracers aimed at detection of amyloid in living patients. Both dyes are relatively planar, hydrophobic aromatic molecules that interact with and bind to the cross-β sheet structure found in all amyloid fibrils. They are unable to cross the blood-brain barrier at least in part due to their ionic charge.
Over the last decade there has been significant progress in the development of positron emission tomography (PET) imaging agents to characterise Aβ plaque burden. PET is a non-invasive molecular imaging technique that relies on the detection of radioactivity emitted from a positron-emitting isotope that is incorporated into a molecular tracer or imaging agent. The emitted positron annihilates releasing two gamma photons travelling in opposite directions. Detection of the emitted photons allows the generation of an image with a spatial resolution of 3-5 mm with high sensitivity. An analogue of ThT, the 2-phenylbenzothiazole derivative 2-(methylamino)phenyl)benzothiazol-6-ol (\(^{11}\)C-PiB) (Figure 6), contains the positron emitting isotope carbon-11, is able to cross the blood brain barrier, and labels Aβ plaques *in-vivo*. It has been used in pioneering clinical studies that have demonstrated the tremendous potential of PET imaging in assisting AD diagnosis. The longer half-life of fluorine-18 when compared to carbon-11 is more practical and fluorine-18 is currently the most widely used radionuclide for PET imaging. Benzothiazole derivatives and an aromatic fluoro-substituted pyridinyl benzofuran radiolabeled with fluorine-18 are in the late stages of clinical development. The pyridynl benzofuran compound, AZD4694 (Figure 6), has completed phase III clinical trials and compares favourably to PiB displaying relatively low non-specific binding when compared to other \(^{18}\)F radiotracers. Flutemetamol (Figure 6), a benzothiazole derivative, has recently gained FDA approval to estimate beta amyloid neuritic plaque density. Another pharmacophore that has shown considerable potential in binding to Aβ plaques contain diaryl alkenes such as stilbenes and styrylpyridines. The development of the \(^{18}\)F labelled stilbenes and styrylpyridines is presented in a 'perspective' article and the research in this area has led to the recent FDA approval of \(^{18}\)F-AV45 (florbetapir) (Figure 6) to detect the presence of amyloid. The use of the benzothiazole dye ThT as a fluorescent probe for the detection of amyloid-like structures was first reported in 1959. In protic solvents ThT absorbs at a wavelength of 340 nm with a relatively weak emission at 445 nm but when bound to amyloid fibrils the dye undergoes a 115 nm red shift in excitation profile \((\lambda_{ex} = 445\ nm)\) with enhanced emission at a wavelength of 485 nm. In aqueous solutions rapid rotation about the C-C bond linking the benzothiazole group to the N,N-dimethylaniline portion of the molecule provides a pathway for non-emissive decay of the excited state. This occurs by a twisted internal charge-transfer process (TICT) from a locally excited state (LE) by increasing the torsion angle from 37° to 90°. The interaction of ThT with amyloid fibrils restricts rotation about the C-C bond and is partially responsible for the increased emission (Figure 7). It is likely that there are three different binding sites for ThT on Aβ fibrils. The first and second binding sites (BS1 and BS2) have higher binding affinities than the third (BS3) and are located closely and/or overlapping to each other. BS1 occurs every ~35 Aβ monomers while BS2 occurs every ~4 Aβ monomers and low capacity BS3 occurs every ~300 Aβ monomers. Hydrophobic grooves are formed on the β-sheet surface by the side-chains of amino acids, particularly Phe and Val. These hydrophobic grooves propagate along the “twisting” Aβ fibril forming BS1 and BS2. Interaction of ThT with the side-chains of these grooves is thought to be primarily via π-π stacking. The low affinity and less abundant BS3 is present on the β-sheet extremities of the fibril. Simulated molecular dynamics support the proposed groove binding of histological dyes and small-molecule imaging agents to amyloid. The Asp-Phe-Gly-Ala-Ile-Leu-Ser peptide sequence, found within human islet amyloid polypeptide, acts as a useful model for binding simulations of small molecule imaging agents and dyes, including ThT and the related radiotracer \(^{11}\)C-PiB. The simulated fibril construct consists of a double layer of β-sheets, each containing 10 in-register antiparallel β-strands. The amino acid side chains decorate the large faces of the fibril to form grooves on both faces (Figure 8a).
Figure 7. Photophysical properties of ThT. (a) The two planar groups within ThT rotate about the C-C bond with an angle of torsion, \( \Psi \). (b) Fluorescence emission of ThT (red) and ThT with A\( \beta \)42 in phosphate buffer (\( \lambda_{\text{ex}} = 444 \) nm). M. Biancalana and S. Koide, Biochim. Biophys. Acta, Proteins Proteomics, 2010, 1804, 1405,1412. Copyright 2010 Elsevier.

The top face of the fibril has a central groove consisting of isoleucine residues and two neighbouring minor grooves formed with isoleucine on one side and alternating arginine and serine residues on the other. Although the bottom face of the fibril has potential for two grooves, phenylalanine residues leaning toward the alanine residues restrict access to one groove but the small alanine residues allow room for a single small hydrophobic central groove. Amongst the multiple potential binding sites available, the two binding modes with the highest binding affinities are in the hydrophobic grooves of the top and bottom faces. The absolute energetic contributions of binding can be classified as polar or non-polar. The non-polar interactions provided the largest contributions to the interactions and a major proportion can be speculatively assigned to \( \pi-\pi \) interactions. Comparison of the charged ThT and its neutral analogue PiB reveals greater insertion into the hydrophobic grooves for PiB corresponding to a more energetically favourable binding to the fibril (Figure 8b & 8c). It is reasonable to expect this to translate to fibrils formed with other peptides, such as A\( \beta \), due to the similarity across amyloid fibril structure.

5 Toward diagnostic imaging of Alzheimer’s disease with copper radiopharmaceuticals

The positron-emitting isotopes of copper offer considerable potential for diagnostic PET imaging. There are two comparatively short-lived isotopes, copper-60 (\( t_{1/2} = 20 \) min) which is relatively easy to produce with a small medical cyclotron and copper-62 (\( t_{1/2} = 20 \) min), available from a convenient generator. Two longer half-life isotopes, copper-61 (\( t_{1/2} = 3.4 \) h) and copper-64 (\( t_{1/2} = 12.7 \) h) can both be produced in low energy hospital cyclotrons. The low energy of the positron-emission from copper-64 is coupled to a lack of interfering gamma emissions so high quality images are obtained that are comparable to those obtained with fluorine-18. In principle, the rapid and simple incorporation of a radioactive copper isotope into a specifically designed targeting ligand is an attractive alternative to the sometimes challenging covalent incorporation of carbon-11 or fluorine-18 into plaque binding tracers. Such a system needs to be carefully designed as factors such as complexation kinetics, thermodynamic stability and biodistribution need to be considered. A single ligand framework is suitable for the full range of copper isotopes and would serve as a valuable platform to develop versatile A\( \beta \) plaque imaging agents and improve the number of clinical centers that are capable of assessing A\( \beta \) plaque burden in patients.

A family of ligands known as bis(thiosemicarbazones) (btsc), derived from 1,2-diones, can be used as delivery vehicles for...
radioactive copper isotopes as they form stable ($K_a = 10^{18}$) and neutral membrane permeable copper complexes.$^{77,81}$ The copper complex $[^{64}\text{Cu}]^{[\text{atsm}]}$ (Figure 9) is currently undergoing clinical trials in humans for imaging hypoxia in head and neck cancers.$^{82,85}$ It is thought that the hypoxia selectivity of the complex involves the metal ion being reduced from Cu$^{II}$ to Cu$^I$. The copper(II) bis(thiosemicarbazidato) complex with a single methyl substituent on the backbone of the ligand, $[^{64}\text{Cu}]^{[\text{gtsm}]}$ (Figure 9), has been used to image blood perfusion in humans and over 5% of the injected dose reaches the brain highlighting its ability to cross the blood brain barrier.$^{86,87}$

![Figure 9. Structures of bis(thiosemicarbazidato) copper(II) complexes.](image)

$[^{64}\text{Cu}]^{[\text{atsm}]}$ is also able cross the blood-brain barrier and the uptake of radiolabelled $[^{64}\text{Cu}]^{[\text{atsm}]}$ in the brain has been used to probe cellular redox status in the brains of patients with mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) and to evaluate striatal oxidative stress in patients with Parkinson's disease. The Cu$^{II}$/Cu$^I$ redox potential of $[^{64}\text{Cu}]^{[\text{btsc}]}$ complexes is dependent on the substituents on the diimine-like backbone of the ligand. For example, the Cu$^{II}$/Cu$^I$ reduction potential in $[^{64}\text{Cu}]^{[\text{atsm}]}$ is about 160 mV more negative than the complex with two hydrogen atoms on the backbone, $[^{64}\text{Cu}]^{[\text{gtsm}]}$. This difference in redox chemistry has been used to investigate differences in copper metabolism in a mouse model of amyloid pathology. The Aβ$_{42}$ peptide binds copper and amyloid plaques have elevated levels of copper (as well as zinc and iron) and it has been suggested that the transmembrane amyloid precursor protein may play a role in the control of nutrient copper. Both $[^{64}\text{Cu}]^{[\text{atsm}]}$ and $[^{64}\text{Cu}]^{[\text{gtsm}]}$ cross the blood-brain barrier but the brain uptake of $[^{64}\text{Cu}]^{[\text{gtsm}]}$ in APP/PS1 model of amyloid pathology mice is higher than $[^{64}\text{Cu}]^{[\text{atsm}]}$. The uptake of $[^{64}\text{Cu}]^{[\text{gtsm}]}$ in the brain (expressed as % of the injected dose per gram (body weight), %ID/g) is significantly higher ($p = 0.01$) in APP/PS1 mice when compared to control animals ($3.0 \pm 0.25\%\,\text{ID/g}$ in the APP/PS1 mice compared to $1.58 \pm 0.14\%\,\text{ID/g}$ in wild type control animals). This difference is presumably a result of changes to copper metabolism and is not a measure of plaque burden.$^{81}$ The ability to alter both the Cu$^{II}$/Cu$^I$ reduction potential and the biodistribution of [Cu$^I$(btsc)] complexes by altering the substituents on the ligand means they have potential to investigate both redox status and altered copper metabolism in other neurological conditions such as Wilson's and Menkes disease. The potential of [Cu$^I$(gtsm)] and related complexes to probe molecular changes in Alzheimer's disease and other neurological diseases warrants more detailed investigations.

As [Cu$^I$(atms)] has sufficient stability for brain imaging it appeared to be a useful starting framework to develop bifunctional chelates with the potential to be used as Aβ plaque imaging agents. The Cu$^I$/Cu$^I$ reduction potential in [Cu$^I$(atms)] is such that it is thought, at least to some extent, reduction is only likely to occur intracellularly in hypoxic cells so reduction to Cu$^I$ would not be expected when targeting extracellular Aβ plaques. A new bis(thiosemicarbazidato) ligand with an appended plaque targeting trans-stilbene functional group, H$_3$L$_1$ was prepared by a condensation reaction between trans-stilbene aldehyde and the bifunctional chelate atsm/a (Figure 9). The copper complex, [Cu$^I$L$_1$], interacts with synthetic Aβ$_{42}$ fibrils in vitro and binds selectively to Aβ plaques in post-mortem samples of human brains from AD subjects (Figure 10). The binding of [Cu$^I$L$_1$] to Aβ plaques was demonstrated in serial sections (7 µm) of frontal cortex tissue treated with a solution of [Cu$^I$L$_1$]. The complex is fluorescent due to the presence of the stilbene functional group and tissue allowing examination by epifluorescence microscopy and comparison to the contiguous section that was immunostained with an Aβ antibody (1E8) to identify plaques. Aβ plaques are typically 40–60 µm in diameter, indicating that 7 µm serial sections would comprise the same Aβ plaques. [Cu$^I$L$_1$] complex crosses the blood–brain barrier and displays increased uptake in the APP/PS1 model of amyloid pathology when compared to age-matched control animals. The brain uptake was significantly higher ($p = 0.005$) in the APP/PS1 transgenic mice when compared with wild-type (2.5% ± 0.6%) compared to 1.7% ± 0.6% ID/g at 7 minutes after the injection.$^{88}$

![Figure 10. Serial sections of human brain tissue from AD affected subjects. (a) Sample immuno-stained with 1E8 antibody and (b) sample treated with [Cu$^I$L$_1$].](image)
spectroscopy showed that all the complexes were charge-neutral in aqueous buffer at pH 7.4. The ligands form stable complexes with 

\[ \text{Cu}^{II} \] and for \[ \text{Cu}^{II} \text{L}_4^2 \] the conditional dissociation constant \( K_D \) is \( 5.8(4) \times 10^{-18} \) M at pH 7.4. The \( \text{Cu}^{II} \) complexes that feature the styrylpyridine functional group, \[ \text{Cu}^{II} \text{L}_3^2 \] and \[ \text{Cu}^{II} \text{L}_4^2 \], have a quasi reversible reduction at about -0.68 V (vs SCE) attributed to a \( \text{Cu}^{II}/\text{Cu}^{I} \) couple, 80 mV more negative the \( \text{Cu}^{II}/\text{Cu}^{I} \) couple for \[ \text{Cu}^{II} \text{L} \]. The complexes \[ \text{Cu}^{II} \text{L}_{3,4}^2 \] bind to Aβ plaques in AD affected human brain tissue, with plaques identified by staining the contiguous section with 1E8 antibody (Figure 11c), even though the pyridyl nitrogen atom within the styrylpyridine is coordinated to \( \text{Cu}^{II} \). Both \[ \text{H}_2 \text{L}_3 \] and \[ \text{H}_2 \text{L}_4 \] formed complexes with copper-64 at room temperature, \[ \text{[Cu}^{II} \text{L}_2^2 \text{]} \] and \[ \text{[Cu}^{II} \text{L}_4^2 \text{]} \], and the distribution coefficients \( \log D \) values of 1.90 and 1.87 are comparable to \[ \text{[Cu}^{I} \text{L} \text{]} \] (1.85). The brain uptake of \[ \text{[Cu}^{II} \text{L}_3^2 \text{]} \] was investigated by micro-PET imaging in wild-type mice and revealed essentially no radioactivity evident in the brain five minutes post injection. The images obtained following administration of \[ \text{[Cu}^{II} \text{L}_4^2 \text{]} \] were more promising with significant radioactivity observable in the brain. A separate biodistribution study that revealed that, at five minutes post injection, 1.11 (0.20) %ID/g of \[ \text{[Cu}^{II} \text{L}_4^2 \text{]} \] had accumulated in the brain, and in wild-type mice without Aβ plaques, the radioactivity cleared to 0.38 (0.09) %ID/g 30 min post injection. The addition of a \( -(\text{CH}_2)_2\text{N(}\text{CH}_3)_2 \) functional group to form \[ \text{[Cu}^{II} \text{L}^2 \text{]} \] increases the molecular weight and has little effect on the \( \log D \) but increases the ability to cross the blood-brain barrier when compared to \[ \text{[Cu}^{II} \text{L} \text{]} \]. This functional group is capable of forming an intramolecular hydrogen bond and may alter the solvation properties of the complex thus modifying membrane permeability. It is also possible that this functional group reduces other complicating interactions such as those with serum proteins or the p-glycoprotein efflux system. Further studies on these systems are warranted including investigation in transgenic models of amyloid and more detailed characterisation of their biodistribution and metabolism. Specifically designed ligands to probe Aβ plaque burden such as the systems presented above could be incorporated into ‘kit’ formulations in a similar manner to successful technetium,\( ^{99m}\)Tc based radiopharmaceuticals. In principle, these ligands would be for suitable each of the positron-emitting isotopes of copper offering incredible versatility and flexibility from a single platform.

**6 Technetium-99m radiotracers designed to bind to amyloid-β**

Although the use of the imaging technique single photon emission computed tomography (SPECT) is routine and widespread, progress toward a SPECT compatible molecular probe has been slower than that of PET. The most commonly used radioisotope for SPECT imaging is technetium,\( ^{99m}\)Tc. This \( \gamma \)-emitting isotope has a half-life of six hours and is available from convenient generator systems. A \( ^{99m}\)Tc-based radiotracer for determining plaque burden would be of considerable clinical utility and increase the number of centres that are able to perform diagnostic scans for AD based on both cost and availability of the necessary infrastructure. As there are no non-radioactive isotopes of technetium, it is common practice to use the relatively stable isotopes of rhenium to form analogues of \( ^{99m}\)Tc complexes for comprehensive characterisation and preliminary *in-vitro* assessment. Detailed reviews on the chemistry of technetium are available and earlier detailed reviews on diagnostic imaging of AD with \( ^{99m}\)Tc complexes were published in 2011 and early 2012 (see Figure 12 for selected examples). The significant progress towards a \( ^{99m}\)Tc complex for Aβ plaque imaging published in articles from 2012 onwards are highlighted in the following section.
A N,S₂ donor often forms neutral complexes with the [M⁹O]₃⁻ (M = Re or Tc) core, particularly the ligands derived from bis(aminoethanethiol) (BAT) and monoamine-monoamide dithiol (MAMA). A comparison between [TcOBAT] and [TcOMAMA] complexes tethered to a phenylbenzoxazole plaque targeting group revealed that the [TcOBAT] complexes displayed higher brain uptake.

Amino, monomethylamino, or dimethylamino substituted pyridyl benzofuran functional groups have also been used as the basis for targeted binding of Aβ plaques in a bifunctional chelate formed by N-alkylation of BAT (Figure 13). Tetradentate N₅S₂ ligands with N-alkylation of the ligand leads to the possibility for syn and anti isomers with respect to the position of the pendant group in relation to the apical oxo ligand. However, in the case of H₅L₅ a single radioactive peak was observed by radio-high performance liquid chromatography upon radiolabeling with ⁹⁹mTc. The octanol water partition coefficient (P) is used to give an indication of the biodistribution of compounds. LogP values were 0.68, 1.35, and 2.09 for ⁹⁹mTcOBAT and ⁹⁹mTcOMAMA respectively. Brain uptake in ‘normal’ mice 2 min post injection was 1.59 (0.21) %ID/g and 1.80 (0.16) %ID/g for ⁹⁹mTcOBAT and ⁹⁹mTcOMAMA respectively while ⁹⁹mTcOBAT has a brain uptake of 1.64 (0.27) %ID/g at 10 min with all three showing good clearance from the brain. The highest brain uptake was shown by ⁹⁹mTcOMAMA (1.80% ID/g) but, of the rhenium analogues, [ReOL]⁺ displayed the highest binding affinity toward Aβ₄₂ aggregates in vitro (Kᵣ = 13.6 nM). The compound that displayed the highest uptake in the brain, ⁹⁹mTcOBAT, was evaluated in Tg2576 mice which display high Aβ plaque deposition in the brain. The murine brain samples examined by ex vivo autoradiography demonstrated that the tracer accumulated in the same deposits as Thioflavin S.

In an alternative approach a dibenzylideneacetone derivative, containing a dimethylamino group, was conjugated to both BAT and MAMA (Figure 14). Once again, rhenium complexes were used as non-radioactive surrogates to assess the binding affinity of complexes toward aggregated Aβ. The binding affinities of MAMA rhenium complexes, [ReOL]⁺, to Aβ₄₂ aggregates were moderate (Kᵣ = 121 and 60 nm for [ReOL]⁺ respectively) increasing to high affinity for BAT rhenium complexes (Kᵣ = 25 and 14 nm for [ReOL]⁺ respectively). All complexes, [ReOL]⁺, bind in vitro to Aβ plaques within brain samples from transgenic model mice (APP/PS1). Complexes ⁹⁹mTcOBAT and ⁹⁹mTcOMAMA display relatively high lipophilicity, with Log D values ranging from 3.17 to 3.53. Brain uptake of the complexes in wild-type mice is relatively low with no significant difference between BAT containing complexes, ⁹⁹mTcOBAT and ⁹⁹mTcOMAMA, and the MAMA containing complex ⁹⁹mTcOMAMA, having an uptake ranging from 0.47 to 0.49% ID/g 2 min post injection. An even lower brain uptake of 0.31 % ID/g 2 min post injection was observed for ⁹⁹mTcOBAT. Compounds ⁹⁹mTcOBAT cleared the brain over time (brain₂₅₅/brain₄₅₅ = 6.13, 3.92 and 5.33 for ⁹⁹mTcOBAT respectively) while a significant amount of activity was recorded in the brain 60 min post injection for ⁹⁹mTcOBAT (brain₂₅₅/brain₄₅₅ = 2). The brain clearance is important for radiotracers as fast brain clearance is necessary to remove unbound radiotracer, giving a high signal to noise ratio affording clear images.
coordination of both a mono and bidentate ligand in the one complex.

A benzothiazole pendant group conjugated to a picolyamine monoacetate chelate ligand coordinates in a tridentate manner with $[\text{M}^1\text{(CO)}_3]^+$ ($\text{M} = \text{Re}$ or $^{99m}\text{Tc}$) to give stable, neutral complexes $[\text{M(CO)}_3\text{L}^2]$ (Figure 16). Human brain tissue, collected from subjects diagnosed with AD, when treated with $[\text{Re(CO)}_3\text{L}^2]$ shows binding of the complex to $\alpha$-fibrils. Similarly, $[^{99m}\text{Tc(CO)}_3\text{L}^2]$ also binds to $\alpha$-fibrils but no binding is detected when a shorter acetamide linker is used to connect the benzothiazole group with the chelate. Brain uptake of $[^{99m}\text{Tc(CO)}_3\text{L}^2]$ administered through tail vein injection to Swiss Albino mice showed moderate brain uptake at 1 minute post injection (0.69%ID/g) combined with fast clearance (0.05% ID/g 15 min post injection; 0.02%ID/g 60 min post injection).

The low valent oxidation state of technetium(I) can be stabilized by carbonyl ligands. Pioneering method development has led to the synthesis of $[^{99m}\text{Tc}][\text{CpM(CO)}_3]$ from $[^{99m}\text{Tc}^3\text{O}]$ using sodium boronocarbonate as both reducing agent and an in situ source of carbon monoxide allowing for the convenience of a kit formulation.108-102 These low spin d$^9$ systems offer high kinetic stability. The stable M-C bonds favour exchange of the water ligands allowing the introduction of mono-, bi- or tridentate ligands and the possibility for [2 + 1] mixed ligand complexes by

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A mixed ligand system, whereby bidentate and monodentate ligands are used to complete the coordination sphere about the fac-[M(CO)_3]^+ producing a “2 + 1” system, allows for the possibility of altering pharmacokinetics without modifying the binding motif. The α,β-unsaturated β-diketone curcumin (HL)^24 binds to Aβ plaques and is able to act as a bidentate ligand when reacted with fac-[M(CO)_3]H_2O)^+] (M = Re or ^99mTc). The remaining aqua ligand can be exchanged for imidazole, isocyanocyclohexane, and triphenylphosphine (Figure 18). The large trans effect exerted by triphenylphosphine can lead to further substitution and formation of [Re(CO)_3(PPh_3)L]^24. The substitution chemistry is similar for ^99mTc. The aqua ligand of the intermediate[^99mTc(CO)_3]L]^24(H_2O), is replaced by a phosphine ligand at room temperature to give[^99mTc(CO)_3]L]^24(PPh_3)] in 80% radiochemical yield, with 20% remaining as the intermediate complex. When incubated at 60°C for 20 min, the reaction progresses to form cis-trans[^99mTc(CO)_3]L]^24(PPh_3)]. Both fac-[Re(CO)_3]L]^24(PPh_3)] and cis-trans-[Re(CO)_3]L]^24 bind to Aβ plaques in post-mortem human brain tissue.105

![Figure 19. (a) Sturcture of the ruthenium polpyridyl complex, [Ru(bpy)(dpdz)]^2+. (b) Representation of a molecular dynamics simulation showing binding of [Ru(bpy)(dpdz)]^2+ to an Aβ fibril. The simulation predicts that binding occurs in a hydrophobic cleft formed between Val18 and Phe20. (c) Photoluminescence spectra of [Ru(bpy)(dpdz)]^2+ incubated with Aβ fibrils (red), Aβ fibrils with buffer (black). Inset shows TEM image of fibril fibrils (scale bar = 200 nm). Images adapted with permission from N. P. Cook, M. Ozbir, C. Katsampes, R. Prabhakar and A. A. Marti, J. Am. Chem. Soc., 2013, 135, 10810-10816. Copyright 2013 American Chemical Society.

54 In similar fashion to its interaction with DNA the interaction of [Ru(bpy)(dpdz)]^2+ with Aβ fibrils also results in a change in the polarity of the microenvironment resulting in enhanced luminescence (Figure 19c).108 The use of a metal based luminescent probe with a long-lived excited state has potential advantages over more conventional dyes such as ThT. The relatively long photoluminescence lifetime of [Ru(bpy)(dpdz)]^2+ bound to Aβ fibrils (185 ns) can be used to differentiate between the competitive binding of other molecules with shorter fluorescent lifetimes. The dissociation constant for the binding of [Ru(bpy)(dpdz)]^2+ to Aβ fibrils is 2.1 µM with a binding stoichiometry of 2.6 Aβ monomers per [Ru(bpy)(dpdz)]^2+. Computational methods that combines molecular docking and all atom molecular dynamics simulation predicted that a hydrophobic cleft between Val18 and Phe20 was a likely binding site with the side chains of these two amino acid residues interacting with the dpdz functional group via CH-π and π-π interactions (Figure 19b).109 The versatility and potential of this family of ruthenium complexes as unconventional probes of protein aggregation is highlighted by the use of the 1,10-phenanthroline analogue, [Ru(phen)(dpdz)]^2+ (where phen = 1,10-phenanthroline), a probe to monitor the fibrilisation of α-syn, a protein associated with Parkinson’s disease, in neuroglioma cells.110

8 Metal complexes to inhibit aggregation and toxicity of amyloid-β (Aβ)

Amyloid-β peptides possess moderate affinity for copper(II), copper(I) and zinc(II). The interaction of Aβ with Cu^2+ involves coordination of the metal ion to imidazole residues on His6, His13, and His14 and alters the aggregation and toxicity profiles of the peptide.25, 31, 33, 111-115 The resulting Cu^2+-Aβ complexes are redox active and lead to the production of toxic reactive oxygen
Modification of the CuII and ZnII binding site of Aβ has the potential to mitigate toxicity and inhibit amyloid formation. An innovative approach of the metal binding properties of Aβ is to use metal complexes with kinetically inert metal ions to form stable coordinate bonds with the histidine residues that are involved in coordination to CuII and ZnII. The challenge is to achieve selectivity for the histidine residues and this, in part, has been achieved by using PtII complexes with hydrophobic phenanthroline ligands and two monodentate co-ligands poised for substitution reactions with the N-donors of the imidazole residues of histidine.

The aggregation and neurotoxicity of Aβ is modulated by the interaction of [PtCl2(phen)] (where phen = 1,10-phenanthroline) and similar complexes (Figure 20).116 These PtII complexes target the metal binding site of Aβ by exchange of the monodentate chloride ligands followed by coordination of the PtII ion to the imidazole functional group in the histidine residues to give [PtII(phen)-Aβ]2+ adducts. It is thought that the complexes target the N-terminal domain of Aβ through π-π interactions between the aromatic phen ligand and the aromatic residues of the peptide (Phe4, Tyr10 and Phe19) that span the CuII binding site. The coordination of the [Pt(phen)]2+ complexes to Aβ inhibits the aggregation of the peptide and leads to precipitation of amorphous aggregates rather than amyloid fibrils. The formation of [PtII(phen)-Aβ]2+ adducts results in inhibition of the neurotoxicity of Aβ and protection against Aβ induced synaptotoxicity in mouse hippocampal tissue.117 The non-covalent interactions between the aromatic phen ligand and aromatic residues of amino acids in the Aβ peptide lead to an acceleration in the rate of reaction as demonstrated by the reaction of [PtCl2(phen)] with the shorter variant Aβ16 (t½ ≈ 4 mins) when compared to the platination of comparable N-donor ligands.118 The formation of [PtII(phen)-Aβ]2+ adducts alters the CuII and ZnII binding properties of Aβ16 and thus changes the aggregation and toxicity profiles of the peptide.119 Following the reaction of Aβ28 with a water soluble sulfonated analogue of [PtCl2(phen)], [PtII(4,7-diphenyl-[1,10]phenanthroline disulfonate)], several products containing a ratio of 1:1 platinum to Aβ28 were identified by mass spectrometry. X-ray absorption spectroscopy in combination with theoretical calculations (density functional theory) suggested the major component was a square planar PtII complex, [PtII((4,7-diphenyl-[1,10]phenanthroline disulfonate))(hist)Cl]2+. The EXAFS refinement gave bond lengths of Pt–N(phen), 1.993(5) Å, Pt–N(imidazole), 2.03(1) Å and Pt–Cl and 2.235(7) Å. In contrast to [PtCl2(phen)] and its analogues, the reaction of cis-[Pt(NH3)2Cl2] with Aβ28 results in a mixture of products that primarily involve coordination to the sulfur atom of Met35. Similar mixed RuIII and PtII complexes also prevent the aggregation of Aβ28 as does a cyclometalated PtII complex, [PtCl(dimethylsulfoxide)(2-phenyl-5-methylpyridine)], which binds to Aβ28.120, 121 The binding of the platinum complex to the peptide does not preclude the coordination of CuII to Aβ28 but does result in a complex where the CuII is in a different coordination environment.122 The interaction of this organometallic complex with this shorter form of the Aβ peptide also inhibits ZnII induced aggregation.123 A strategy of combining the Aβ binding ability of platinum complexes containing aromatic ligands with the copper(II) and zinc(II) binding properties of the tetrazamacrocyclic chelator cyclen has led to the synthesis of cyclen-Pt(bpy) conjugates (where bpy = 2,2-bipyridine). Upon interaction with Aβ40 the PtII ion of these conjugates coordinates to His14/13 and the macrocyclic ligand is able to sequester CuII and ZnII. The simultaneous metal chelation and coordinate bond modification of the peptide alter the aggregation of Aβ40.124

Each of these platinum(II) complexes are likely to have poor bioavailability and blood-brain barrier penetration and this has the potential to compromise their clinical development for the treatment of neurodegeneration. The desire to provide orally bioavailable platinum(II) complexes with the potential to alter Aβ aggregation led to the synthesis of a platinum(IV) complex with a specifically designed benzimidazole quinoline ligand (L25) (Figure 21). The idea of using a PtIV complex as a pro-drug activated by in vivo reduction to a PtII species was inspired by the similar approach used to make orally bio-available and less toxic platinum complexes for the treatment of cancer (Satraplatin). Complex [PtIVCl2L25] was administered orally to the APP/PS1 mouse model of amyloid pathology. Analysis of the brain tissue following treatment revealed a 40% reduction in Aβ42 levels and a reduction in Aβ plaques when compared to untreated animals (Figure 21).125

Figure 20. The structures of [PtCl2(phen)], [PtCl(dimethylsulfoxide)(2-phenyl-5-methylpyridine)] and [M(ppy)(OH2)]+ (where M = RhIII or IrIV).

Figure 21. (a) Structure of [PtIVCl2L25]. Representative examples of brain tissue from transgenic APP/PS1 mice immunohistochemically stained for Aβ when (b) untreated or (c) treated with [PtIVCl2L25]. Images adapted with permission from V. B. Kenche, L. W. Hung, K. Perez, I. Volitakes, G. Ciccostoto, J. Kwok, N. Critch, N. Sherratt, M. Cortes, V. Lal, C. L. Masters, K. Murakami, R. Cappai, P. A. Adlard and K. J. Barnham, Angew. Chem., Int. Ed., 2013, 52, 3374-3378. Copyright 2013 John Wiley and Sons.
The potential of metal complexes with kinetically inert $d^6$ complexes to inhibit Aβ amyloid has been extended to Ir$^{III}$, Rh$^{III}$, and Ru$^{III}$ complexes. Iridium(III) and rhodium(III) complexes with orthometallated phenylepyridine (ppy) ligands, $[M$(ppy)$_2$(OH)$_2$]$_2^+$ (where M = Rh$^{III}$ or Ir$^{III}$) (Figure 20), inhibit the aggregation of Aβ$_{40}$ with $[Rh$(ppy)$_2$(OH)$_2$]$_2^+$ the most effective, inhibiting aggregation at concentrations of 5 μM. It is likely that exchange of the aqua ligands is followed by coordination of the metal ion to the imidazole side chains of the histidine residues in Aβ$_{40}$. The complexes formed from $[Ir$(ppy)$_2$(OH)$_2$]$_2^+$ in the presence of Aβ$_{40}$ are luminescent and the emission intensity ($λ_{em} = 491$ nm) is higher for aggregated $[Aβ_{40}-Ir$(ppy)$_2$]$_2^+$ complexes when compared to the monomeric form suggesting this system could be used as an alternative to conventional fluorescent dyes for monitoring fibrilisation. A series of ruthenium(II) complexes also interact with Aβ peptides and pioneering studies have identified some interesting substitution chemistry. The reaction of $fac-[Ru$(CO)$_3$Cl$_2$(1,3-thiazole)] (Figure 22) with Aβ$_{25}$ results in a mixture where the predominant product identified by electrospray mass spectrometry is of the composition $[Ru$(CO)$_3$-Aβ$_{28}$]$_2^+$ and similar complexes were identified when longer Aβ$_{42}$ replaced Aβ$_{28}$. The coordination of the $[Ru$(CO)$_3$]$_2^+$ fragment to Aβ$_{25}$ results in dramatic changes to the $^1H$ NMR spectrum with significant broadening of signals attributed to the three histidine residues and tyrosine-10 implicating the histidine residues in coordination to the Ru$^{III}$. A ruthenium(III) monoanionic complex with two 2-aminothiazole N-bound ligands and four chloride ligands (PMRU20) (Figure 22) was found to offer protection against Aβ induced toxicity in rat primary cortical neurons. Interestingly two other structurally related Ru$^{III}$ complexes, NAMI A and KP1019 (Figure 22), offered little to no protection. The difference in reactivity between these complexes is intriguing and warrants further investigation. The reaction of selected ruthenium complexes with proteins involved in the pathology of type II diabetes (human islet amyloid polypeptide) and prion diseases (PrP$_{106-126}$) also results in significant changes to the aggregation of the respective proteins. This further highlights the potential of coordinate bond formation with kinetically inert metal ions to act as novel inhibitors of amyloid formation.

![Figure 22. Structure of ruthenium complexes whose reaction with Aβ peptides have been investigated.](image)

**Conclusions**

The full details of the role Aβ plaques, oligomers, tau and neurofibrillary tangles play in cognitive impairment in Alzheimer’s disease remain a mystery. What is known about the extracellular amyloid deposits evident in the cortex of subjects with the disease is that they contain relatively high concentrations of aggregated amyloid-β protein, a 39-43 amino acid fragment derived from the amyloid precursor protein. Our understanding of the structure and actual function of both Aβ and APP remains incomplete. It is possible that there is still much to learn about the generation, clearance and toxicity of Aβ as well as the molecular dynamics involving Aβ monomers, oligomers and plaques. Significant progress in the last decade has led to the development of molecular tracers that can offer insight into the deposition of amyloid in the brain of living patients using non-invasive PET imaging. The results of pioneering multi-centre studies using PET tracers to detect amyloid in large patient cohorts are emerging and providing valuable insights into the relationship between amyloid and cognition.

There are several radioactive isotopes of copper that have the potential to be of use in PET imaging and technetium-99m continues to be the radioisotope of choice for SPECT imaging. In principle, ligands designed to bind to amyloid plaques and form stable copper or technetium complexes have the potential to extend the capabilities of amyloid imaging. A new ligand based on a bis(thiosemicarbazone) framework with appended plaque targeting functional group and hybrid thiosemicarbazone-styrylpyridine ligands have shown some promise as ligands for copper radiopharmaceuticals. A wide range of Te complexes designed to bind to Aβ plaques is discussed in the review. The “piano stool” complex, $[^{99m}$Tc$^{II}$(figure 21), shows significant potential and warrants further investigation.

Luminescent ruthenium and iridium metal complexes with long fluorescent lifetimes that undergo significant changes to their electronic spectra upon binding to Aβ fibrils have potential as useful probes to gain increased insight the molecular aspects of amyloid formation and aggregation. The interaction of the hydrophobic complex $[Ru$(bpy)$_3$(dppz)]$_2^+$ with Aβ$_{40}$ results in a dramatic change in the photoluminescence of the complex and the long fluorescent life-time could be of use in competitive binding assays with other small molecules. An innovative approach at altering the metal-binding properties, toxicity and aggregation profiles of Aβ is to use metal complexes of kinetically inert metal ions to form coordinate bonds to the histidine residues in the metal-binding domain of Aβ. Complexes with the kinetically inert ions, Pt$^{II}$, Ru$^{III}$, Ir$^{III}$ and Rh$^{III}$ all interact with Aβ peptides of varying length to give adducts that have altered toxicity and propensity to aggregate. The viability of metal complexes to be therapeutically useful inhibitors of amyloid formation and Aβ toxicity will be dependent on them satisfying the stringent and challenging requirements of therapeutic agents.

**Notes and references**

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2. [Figure 22. Structure of ruthenium complexes whose reaction with Aβ peptides have been investigated.](image)