Neurochemistry

DOI: 10.1002/anie.201209885

Development of a Platinum Complex as an anti-Amyloid Agent for the Therapy of Alzheimer's Disease

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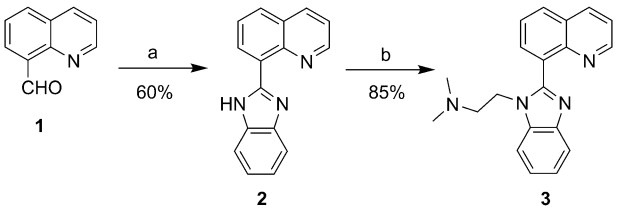
Alzheimer's disease (AD) is an age-related neurodegenerative disease. Its pathological indicators include extracellular amyloid plaques, the main constituent of which is the amyloid β-peptide (Aβ), and neurofibrillary tangles composed of hyperphosphorylated tau protein.[1] Current evidence suggests that the aggregation of Aβs drives the disease process, as various forms of aggregated Aβ have been shown to be toxic,[2] resulting in the development of a variety of therapeutic strategies that target Aβ.[3–6] To date, most Aβ aggregation inhibitors have been designed to target the hydrophobic central and C-terminal regions of Aβ, which are in general conjugated polyaromatic molecules that are very hydrophobic.[7] Herein, we report a different approach to the design of aggregation inhibitors of Aβ and demonstrate that this approach can modify Aβ in vivo. Aβ contains a metal-binding motif with three histidine residues (6, 13, and 14) near the N terminus, and the interaction of this site with zinc and copper modulates the aggregation and toxicity of Aβ.[8,9] We have previously taken advantage of the metal-binding ability of Aβ to show that commercially available Pt^{II} complexes of 1,10-phenanthroline ligands target this site, thus inhibiting Aβ aggregation in vitro.[10] For a variety of reasons, including lack of novelty, cumbersome multi-step synthetic procedures,[11,12] and poor bioavailability, these complexes are unsuitable for in vivo studies. Therefore we sought to identify new Pt complexes that are suitable for in vivo studies.

The 8-(1*H*-benzoimidazol-2-yl)-quinoline (8-BQ)[13] scaffold (Scheme 1) was identified as suitable for generating the desired platinum complexes. The ligand requires few steps to synthesize and provides the large aromatic surface area required for a Pt complex to target Aβ. Surprisingly, although the initial synthesis of 8-BQ was first reported[14] over 100 years ago, the coordination chemistry of this ligand has not been widely explored and Pt complexes of 8-BQ are novel. Additionally, the presence of an NH functionality on the imidazole moiety of 8-BQ allows easy modification of the ligand through a conventional one-step substitution reaction. Attachment of different groups to this position can be used to modulate the solubility and other pharmacokinetic properties of the complexes. We chose the *N*,*N*-dimethylaminoethyl group because it improves drug solubility and stability in aqueous media.[15] Herein, we report the synthesis of the 8-BQ ligand (Scheme 1) and its coordination to Pt^{II} and Pt^{IV} complexes (Schemes 2 and 3, respectively).



Scheme 1. Synthesis of *N*,*N*-dimethyl-2-[2-(quinolin-8-yl)-1*H*-benzimidazol-1-yl]ethanamine (**3**). a) 1,2-Phenylenediamine, 50% aqueous THF, reflux, 24 h, 60%. b) 2-(Dimethylamino)ethyl chloride hydrochloride, Cs_2CO_3, DMF, 80°C, 16 h, 85%. DMF = *N*,*N*-dimethylformamide.

The desired 8-BQ ligand **3** was obtained in two synthetic steps, a condensation between 8-quinolinecarboxaldehyde (**1**)[16] and 1,2-phenylenediamine followed by a conventional base-catalyzed N alkylation of benzimidazole **2**. A 1:3 mixture of **1** and 1,2-diphenylamine was dissolved in an equimolar mixture of THF and water (saturated with O_2 gas) and heated to reflux for 24 hours to give **2** in 60% yield. Here, molecular oxygen was used to form the required benzimidazole moiety by oxidative cyclo-dehydrogenation of an intermediate Schiff's base.[17] The N alkylation was performed by refluxing the mixture of **2**, 2-chloro-*N*,*N*-dimethylethylamine

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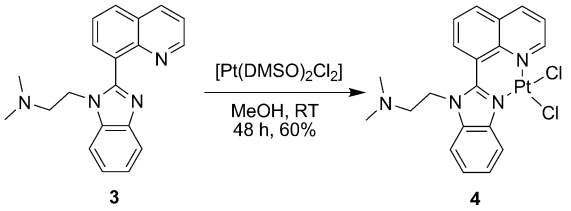
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201209885.

hydrochloride, and Cs_2CO_3 in dimethylformamide for 16 hours to give the desired ligand **3** in 85% yield.

The Pt^{II} complex of **3** was prepared by adopting the reported method for synthesis of Pt(1,10-phenanthrolines) complexes[18] (Scheme 2). Equimolar amounts of **3** and



Scheme 2. Synthesis of [Pt^{II}(*N*,*N*-dimethyl-2-[2-(quinolin-8-yl)-1*H*-benzimidazol-1-yl] ethanamine) Cl_2] (**4**). DMSO = dimethyl sulfoxide.

$[PtCl_2(DMSO)_2]$[19] were stirred in methanol for 48 hours at room temperature to give **4** as a yellow solid in approximately 60% yield. The complex was characterized by ^1H NMR and ^{195}Pt NMR spectroscopy and ESI/MS (see the Supporting Information).

The toxicity of Aβ is linked to peptide aggregation. Time-dependent aggregation of Aβ into fibrillar structures can be monitored by fluorescence of thioflavin T (ThT), which gives a characteristic signal when bound to amyloid.[10] Complex **4** inhibited ThT fluorescence in a dose-dependent manner (Figure 1a), while the uncomplexed ligand **3** had no effect on ThT fluorescence (data not shown). Negative-staining electron microscopy confirmed that **4** inhibited the formation of fibrils (see Figure S1 in the Supporting Information).

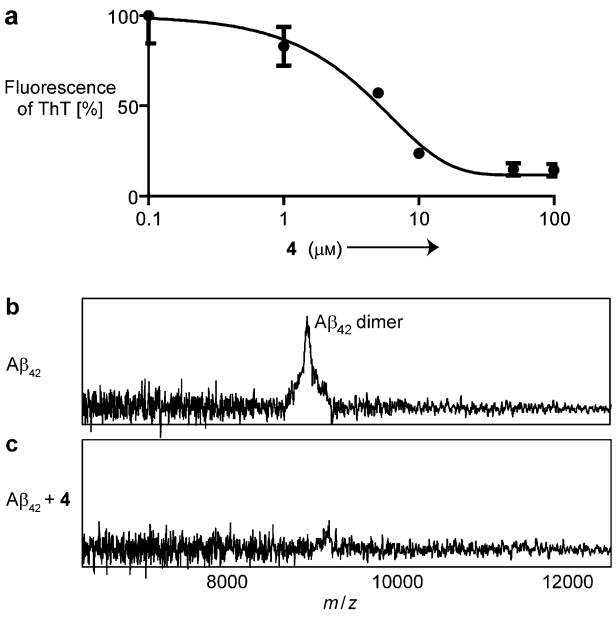


Figure 1. Inhibition of Aβ aggregation. a) Fluorescence emission spectra of thioflavin T (ThT) upon addition of $Aβ_{42}$ (10 μM) incubated with **4** in various concentrations at RT for 24 h. Inhibition of Aβ amyloid formation by **4** was observed in a dose-dependent manner. Error bars: +/− standard deviation. b, c) SELDI-TOF mass spectrum of $Aβ_{42}$ (10 μM) incubated b) with vehicle at RT for 6 h to show the formation of dimeric $Aβ_{42}$, and c) with **4** (10 μM) at RT for 6 h, showing that the dimeric form of Aβ is absent.

Fibrillar amyloid is the end result of the Aβ aggregation process, but it is now thought that Aβ toxicity is due to smaller soluble oligomeric forms of Aβ. We used two methods, SDS-PAGE and surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectrometry to determine whether **4** altered the oligomeric profile of Aβ. In the first instance, Aβ was aggregated in PBS buffer with and without **4** at 37 °C over 20 hours with samples taken periodically and analyzed by SDS-PAGE Western blotting using the 4G8 antibody (epitope residues 17–24 of Aβ; see Figure S1 in the Supporting Information). The blot from Aβ that was aged for 20 hours in the absence of **4** showed extensive "smearing" of immunoreactivity, consistent with peptide aggregation. This "smearing" and therefore the degree of aggregation was significantly reduced by co-incubation of Aβ with **4**. We have previously shown that small oligomeric forms of Aβ can be observed using SELDI-TOF MS and that these oligomeric forms of Aβ correlate with the toxicity of peptides.[20] When Aβ was incubated both with and without **4**, two new signals appeared in the spectrum after 4 hours in the presence of the drug. The signals were shifted from the mass of Aβ (4515 ± 1 Da) by 547 Da and 512 Da, and these differences in molecular weight are consistent with **4** loosing either one or both chloride atoms as the Pt complex binds to Aβ. Significantly, while a signal corresponding to the dimer of Aβ was detectable in the mass spectrum of untreated Aβ, this signal was absent from the spectrum of Aβ incubated with **4** (Figure 1b,c).

After the demonstration that **4** inhibited Aβ aggregation, its ability to inhibit Aβ toxicity in primary mouse cortical neuronal cell cultures was assessed. Treatment of neurons with $Aβ_{42}$ (10 μM) for 4 days reduced cell viability to 77%, as measured by the MTS assay (Figure 2a). Co-incubation of $Aβ_{42}$ (10 μM) with **4** (10 μM) significantly increased cell viability to 94% (Figure 2a). Complex **4** was not toxic at the concentrations tested. Long-term potentiation (LTP) in rodent hippocampal slices is a measure of synaptic plasticity that focuses on activity-dependent persistent increases in synaptic strength, and is considered to be the biochemical basis of learning and memory.[21,22] High-frequency stimulation of a mouse hippocampal slice gives an LTP of (128 ± 6)% (Figure 2b). Incubation of the hippocampal slice with $Aβ_{42}$ (2 μM) for 30 minutes before stimulus significantly reduced LTP to (91 ± 4)% (Figure 2c). Addition of **4** (2 μM) to the solution of $Aβ_{42}$ inhibited its effects on LTP ((133 ± 11)%; Figure 2c).

The ultimate objective of this work was to generate an orally administered anti-amyloid compound for in vivo testing. To prepare an orally bioavailable Pt complex capable of inhibiting Aβ aggregation, a prodrug strategy was adopted, as previously used in the development of JM216 (Satraplatin), an orally bioavailable Pt^{IV} anticancer complex.[23] Substitution reactions for Pt^{IV} complexes are kinetically very slow,[24,25] in a biological context this means the complexes are very stable and able to survive the acidic environment of the stomach and to cross gut membranes intact. Having crossed these mem-

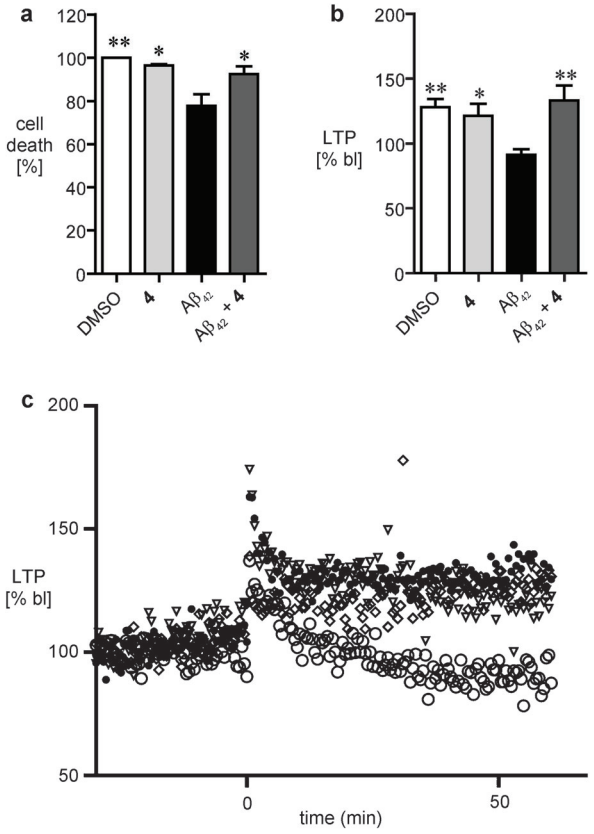
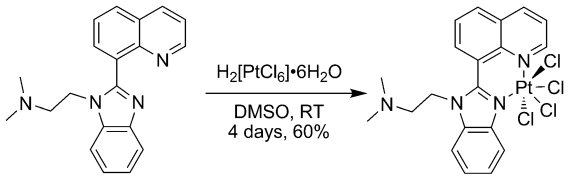


Figure 2. Inhibition of Aβ toxicity. a) Aβ treatment reduces cell viability, which is rescued by co-incubation of Aβ with complex **4**. b) LTP levels quantified as the baseline percentage (% bl) of the field excitatory postsynaptic potential (fEPSP) averaged from 55 and 60 min after tetanic stimulation. c) Rescue of $A\beta_{42}$ synaptotoxicity. When compared with treatment by DMSO vehicle alone (open diamonds, ◇), long-term potentiation (LTP) was significantly inhibited by treatment with $A\beta_{42}$ (2 μM; open circles, ○). Treatment of control slices with **4** (2 μM; open triangles, ▽) alone did not affect LTP, but inhibition of LTP by $A\beta_{42}$ was completely reversed by co-incubation with **4** (2 μM; closed circles, ●). *$p < 0.05$, **$p < 0.01$.

branes, the prodrug is then reduced by natural reductants, such as glutathione, to the active Pt^{II} complex.[23]

Generally, Pt^{IV} complexes are prepared by oxidation of Pt^{II} complexes utilizing agents such as Cl_2[26] and H_2O_2,[27] but in this instance, attempts to oxidize **4** failed as the 8-BQ ligand was prone to oxidative degradation. Therefore, a direct reaction of equimolar amounts of **3** with H_2PtCl_6 in DMSO at room temperature for 4 days was adopted, giving the desired product **5** in 60% yield (Scheme 3). Attempts to use higher temperatures to shorten the reaction time led to an intractable mixture of compounds. Complex **5** was characterized by ^1H NMR and ^{195}Pt NMR spectroscopy and ESI/MS (see the Supporting Information).

To ensure that the Pt^{IV} prodrug strategy results in higher levels of bioavailable drug, we treated wild-type mice with either **4** or **5** at 15 mg kg^{-1} (note that on a molar basis, the animals treated with **5** received slightly less drug). Blood and brain tissues were collected at 5, 30, and 60 minutes post dosing, plasma was separated from the blood, and the brain



3 **5**

Scheme 3. Synthesis of [Pt^{IV}(*N*,*N*-dimethyl-2-[2-(quinolin-8-yl)-1*H*-benzimidazol-1-yl] ethanamine) Cl_4] (**5**).

tissue homogenized. As a surrogate measure of drug levels, plasma and brain homogenates were analyzed for levels of Pt by inductively coupled plasma mass spectrometry (ICP-MS). As predicted, the levels of Pt in the plasma were significantly higher in the animals treated with **5** than **4** ((1.7 ± 0.8) and (0.3 ± 0.1) μmol of Pt, respectively) after 60 minutes. The higher level of Pt in the blood was also reflected by higher levels of Pt in the brain after 60 minutes ((0.006 ± 0.002) and (0.003 ± 0.001) μg of Pt per g of body weight). These data are consistent with the Pt^{IV} prodrug being more bioavailable.

To ensure that **5** can be reduced to the active Pt^{II} complex in vivo, **5** was mixed with glutathione, and the reduction of Pt^{IV} to Pt^{II} was monitored with ^{195}Pt NMR spectroscopy. The ^{195}Pt chemical shift of **5** is −316 ppm, typical of a Pt^{IV} complex. When mixed with glutathione for 20 minutes, this resonance disappeared and a new resonance was visible at −2365 ppm, consistent with a Pt^{II} species (see the Supporting Information).

We initially tested **5** at a dose of 15 mg kg^{-1} d^{-1} by oral gavage for 18 weeks in the Tg2576 mouse model of AD.[28] We found that treatment with **5** reduced the number of Aβ plaques by 29%, although this result did not reach statistical significance (see Figure S2a in the Supporting Information). While there was no change in Aβ levels detected by Western blot, when the same tissue was analyzed by SELDI-TOF MS using the 4G8 antibody (epitope residues 17–21 of Aβ), the changes in the spectra of treated and untreated animals were quite pronounced (see Figure S2b and c in the Supporting Information). Perhaps most significantly, signals at 9260 and 12200 Da, which were visible in the vehicle-treated group, were significantly reduced by treatment with **5** (see Figure S2d and e in the Supporting Information). Species with these approximate molecular weights have previously been implicated as "naturally derived" toxic forms of Aβ.[29]

Next we tested **5** in the APP/PS1 mouse model of AD.[30,31] Analysis of brain tissue using SELDI-TOF MS showed that treatment led to a statistically significant 40% reduction in $A\beta_{42}$ levels ($p = 0.025$; Figure 3a,b). This result was accompanied by a statistically significant 26% decrease in plaque number in the treated animals ($p = 0.01$; Figure 3c,d,e). Western blotting indicated that treatment resulted in a decrease of Aβ levels in the insoluble fraction within the brain, although this observation did not reach significance.

Deposition of Aβ in the brain is one of the defining features of AD, and the peptide has thus been the major target of therapeutic strategies devised for AD. Herein, we set out to develop a novel Pt complex capable of being administered orally and reducing the amyloid burden in the

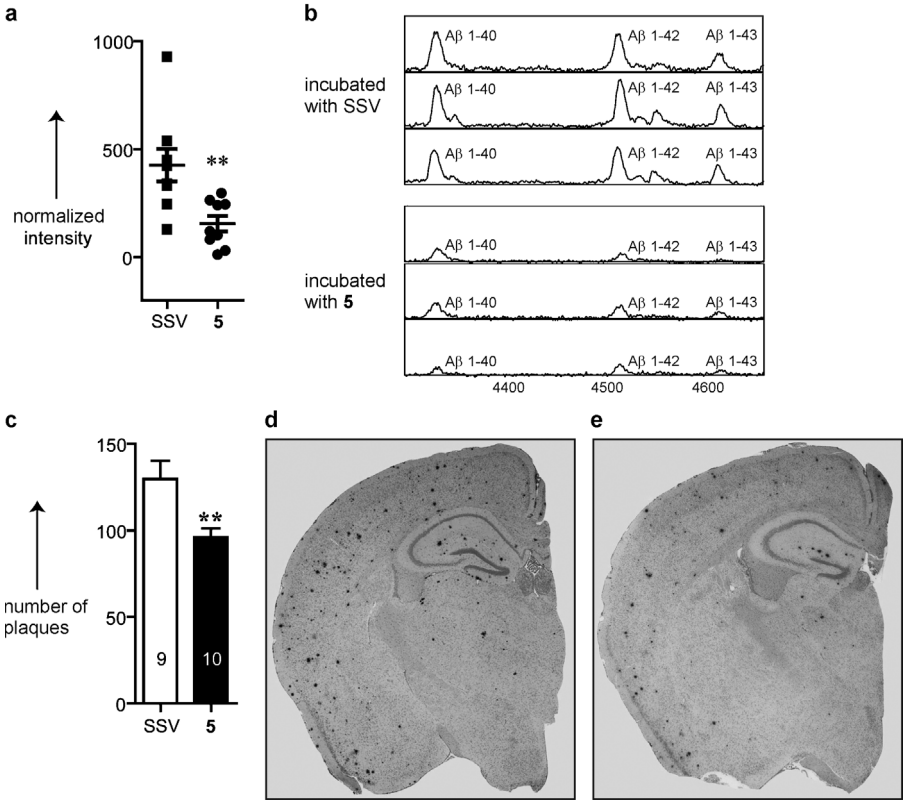


Figure 3. Reduced Aβ levels in APP/PS1 mice after treatment with **5**. a) Analysis of brain tissue by SELDI-TOF MS showed that treatment with **5** lowered levels of Aβ in the brains of APP/PS1 mice as compared to vehicle-treated mice. Representative mass spectra are shown in b. c) Quantitation of plaque number shows that treatment with **5** reduced plaque numbers compared to vehicle-treated control mice. Representative examples of the immunohistochemical staining for Aβ in d) vehicle-treated mice, and e) mice treated with **5**. SSV = standard suspension vehicle.

brains of transgenic mouse models. The data presented here shows that we successfully achieved this goal. However, more work is required to ascertain the full extent to which Pt complexes may be useful as potential AD therapeutics. Recent results from clinical trials of anti-Aβ immunotherapy approaches have indeed suggested that merely reducing the amyloid burden in the brain of patients suffering from AD is insufficient to improve cognitive function. It now appears as though amyloid deposition may begin up to 20 years before a patient presents any clinical symptoms of the disease.[32,33] As such, many researchers now believe that treatment of patients with anti-amyloid drugs at the mild to moderate stage of disease is too late, and that for anti-amyloid approaches to be successful, treatment must begin early in the disease process, either at the prodromal stage, at which patients show mild cognitive impairment, or if patients can be identified, treatment should ideally begin at the preclinical stage of disease. The treatment regime utilized in this study involved treating relatively older animals with established amyloid burden. While these data suggest that the Pt compound impacted the natural course of pathology in the animal models, we have yet to ascertain whether there is any added benefit of treating early in the disease process. This study, however, supports the exploration of the use of novel Pt compounds in the treatment of AD.

Received: December 11, 2012
Published online: February 10, 2013

Keywords: Alzheimer's disease · amyloid beta-peptides · neurochemistry · platinum · quinolines

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