The Amide Derivatives of Chrysamine G Bind to the β-Amyloid Fibril

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The pathological hallmark of AD is a deposition of amyloid plaques in the brains of patients. β-amyloid protein is the major protein component of Alzheimer's plaques. When aggregated into amyloid fibrils, the peptide is toxic to neuronal cells.[1] The neurotoxicity of Aβ fibril is closely related to the β-sheet conformation and aggregation of Aβ peptide.[2] Various compounds which have affinity for β-amyloid fibril have shown ability of preventing neurotoxicity of β-amyloid fibril and inhibiting aggregation of β-amyloid fibril.[3] Therefore, the development of new compounds which have affinity for the β-amyloid fibril would lead to the new compounds that could have therapeutic effects on AD. In addition, these amyloid fibril-philic molecules could lead to new diagnostic molecules for *in vivo* quantification of amyloid deposition[4] and can be used as probes for amyloid structure.[5] Among the known amyloid-philic compounds, Congo Red and Chrysamine G are famous and have similar structures (Fig. 1). They both have the ability to protect neuronal cells from the toxicity of β-amyloid fibril and Congo Red can inhibit the aggregation of β-amyloid peptide.[6] Therefore, Congo Red and Chrysamine G mimicking compounds would lead to diverse new compounds which would have similar or even better properties than Congo Red and Chrysamine G. For the generation of the Congo Red and Chrysamine G based chemical library, we analyzed the structures of Congo Red and Chrysamine G to make more diverse analogues from them. The structures of Congo Red and Chrysamine G can be analyzed into the central spacer connected to the same side arms (Fig. 1). Variation of the spacers and the side arm would lead to diverse structures based on the structures of these molecules. However, diazo bonds, which are connecting central spacer and two side arms, seem to be inconvenient functional group to be used for the generation of diverse structures. This diazo group might be changed to amide group as amide group has double bond character and the generation of amide bond is relatively easy compared to the generation of diazo bond. Before we generate the diverse structures with amide bonds, we investigated the influence of exchanging diazo bond with amide bond to the binding affinity to the β-amyloid fibril. For simplicity, we have chosen Chrysamine G as a model system. As the phenolic alcohol group does not affect the binding affinity to the β-amyloid fibril,[7] it was removed for further simplicity. Based on these ideas, we generated three analogues of Chrysamine G and all of them showed high binding affinity toward β-amyloid 1-40 fibril. The synthesis of these compounds are shown in Figure 2 As lithiated salt form, they were readily soluble in water. For the binding study, 80 μL of various concentrations of the amide analogues of Chrysamine G in water (5-100 μM) were added to

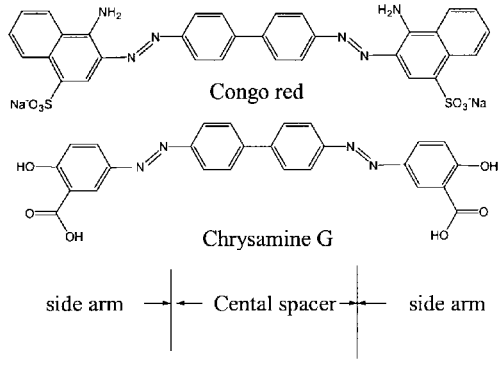

Figure 1. The structures of Congo Red and Chrysamine G.

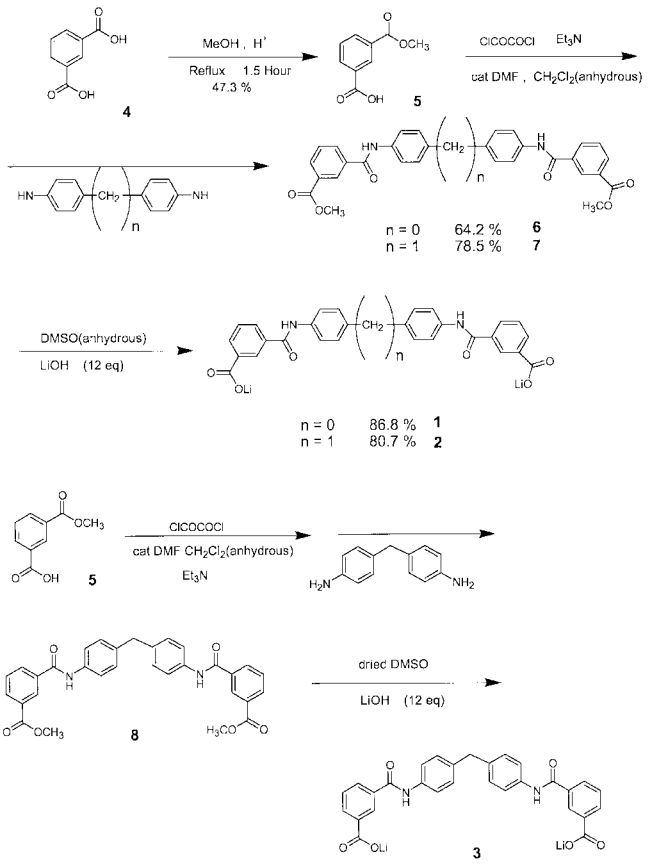

Figure 2. The Synthesis of amide derivatives of Chrysamine G.

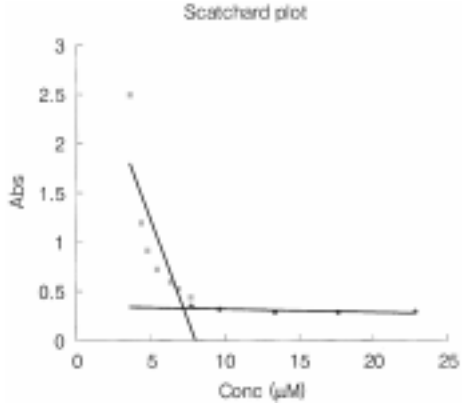

Figure 3. The Scatchard analysis of Compound **1**.

the 20 μL of 100 μM β-amyloid 1-40 fibril. After 2 hours at 37 °C, the incubating eppendorf tubes were centrifuged (16,000 rpm, 20 min) to spin down the β-amyloid fibril and the compounds bound to the β-amyloid fibril. Then the concentrations of unbound compounds in the solution were measured by UV spectrometer. Figure 3 shows Scatchard analysis of the binding of **1** to the β-amyloid 1-40 fibril. Like Chrysamine G, **1** has two binding sites. The higher affinity binding site appears to have a K_d of 1.91 μM and a Bmax of 0.42 moles per mole of amyloid 1-40 peptide. The lower affinity binding site appears to have a K_d of 180.77 μM and a B_{max} of 3.75 moles per mole of β-amyloid 1-40 peptide. As the structure of the β-amyloid fibril is not clear, a systematic variation of the structure to investigate structure activity relationship is reasonable approach. The compound **2** and **3** are synthesized to see the effects of the modification of central spacer and the position of side arm. They also show two binding sites and their K_d and B_{max} are shown in Figure 4 along with those of Chrysamine G. The higher affinity binding sites of compounds **1**, **2** and **3** are well defined as they have very similar values of B_{max}. However, the lower binding sites are not well defined. Klunk *et al.* proposed the electrostatic interaction model between the anionic groups of the Chrysamine G and regularly spaced cationic groups at the amyloid surface.[5a] Our result is not consistent with the electrostatic model as the distances of carboxylic acid in compounds **1**, **2** and **3** do not match to either 5 peptide chains (19.1 Å = 4 × 4.76) or 4 peptide chains (14.28 Å = 3 × 4.76). The distances between carboxylic acid of the compound **1**, **2** and **3** are 17.6, 17.2 and 16.1 Å (Cache3.1 MM2 Energy Minimization), which is between 19.1 Å and 14.28 Å. Cooper suggested Congo Red binding to the channels of the β-amyloid fibril backbone.[8] If the compound **1**, **2** and **3** bind to specific channels of the β-amyloid fibril backbone, hydrophobic interaction might be important inside the channels as the compounds **2** and **3** has higher binding affinity than the compound **1**.

Finally, the binding mode of the compound **1**, **2** and **3** is not clear yet. However, the exchange of diazo bond with amide bond in the Chrysamine G backbone is tolerated. The detailed modeling for the binding mode of these compounds and their potencies in protecting neuronal cells from the toxicity of β-amyloid fibril are currently under investigation.

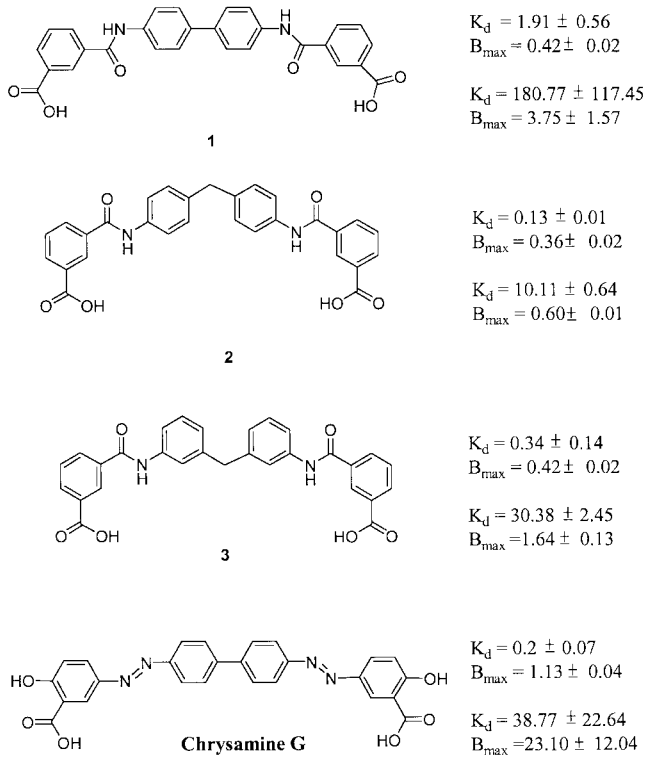

Figure 4. K_d and B_{max} values of the compound **1**, **2**, **3** and Chrysamine G.

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