Considerable evidence supports that deposition of β-amyloid peptide is closely related to neurodegeneration in Alzheimer’s disease. Aggregated β-amyloid peptide with β-sheet conformations is toxic to a variety of cell types in culture. These findings have led to the search for the chemical compounds which would prevent Aβ aggregation and Aβ-induced neurotoxicity. 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. Among the known amyloidophilic compounds, Congo Red and Chrysamine G have high affinity to the β-amyloid fibril and ability to protect neuronal cells from the toxicity of β-amyloid fibril. As they have similar structures, mimicking their structures could lead new compounds with similar or even better activity. Recently, we have synthesized compounds which mimicked the structure of Chrysamine G and showed they had similar affinity to β-amyloid fibril with Chrysamine G. The key structural difference between Chrysamine G and their mimicking compounds is that the diazo bonds of Chrysamine G are replaced by amide groups for the generation of more diverse structures (Figure 1).

In this study, we would like to report that Chrysamine G mimicking compounds 1 and 2 protect human astrocyte cells against Aβ-induced toxicity. We examined the ability of the compound 1 and 2 to modulate the cellular toxicity of aged Aβ 1-40 using an MTT reduction assay. Cell reducing activity is a measure of mitochondrial function. A decrease in ability to reduce MTT is an early indicator of Aβ mediated toxicity. In experiments, suitable amounts of compounds 1 and 2 in deionized water were incubated with aggregated Aβ in PBS buffer at room temperature for 24 hours. Then this chemically treated Aβ 1-40 fibril were added to human astrocyte cell which were maintained in DMEM/F12 medium containing 10% fetal calf serum, 100 units/mL penicillin and 100 g/mL streptomycin. The final volume of total solution was 100 μL and the final concentration of Aβ 1-40 were 10 μM. After human astrocyte cell and chemically treated Aβ 1-40 incubated for 24 hours, the tetrazolium salt MTT [3-(4,5-demethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide] was added to the cell culture and incubated further for 3 hours at 37°C. Formazan, the end product of MTT assay, was dissolved in DMSO and the optical density was measured at 570 nm using ELISA microplate reader.

Incubation of human astrocyte cells in brain with 10 μM Aβ 1-40 fibril resulted in about 70% inhibition of MTT reduction. Figure 2 demonstrates the protective effect of
compound 1 and 2 against Aβ 1-40 fibril induced reduction of cellular redox activity of human astrocyte cells. A significant protective effects of compound 1 and 2 were seen at 100 µM. The protective effects are increased when the concentrations of 1 and 2 are increased to 250 µM. At this concentration, 2 seemed to show complete protection of cells. The effect was not increased when the concentrations of 1 and 2 were increased to 500 µM. In case of compound 2, about 20 equivalents of the compound 2 were necessary for the complete protection of human astrocyte cells. The potency of 2 is similar to Congo Red. 11-13 However, the potency of 2 is lower than Chrysamine G.

The mechanism of protective effect of 1 and 2 is unknown. Klunk suggested that highly conjugated nature of Chrysamine-G and salicylic acid moieties could prevent the oxidative/free radical actions of Aβ. 8 In our compounds the conjugation is destroyed by removing diazo bonds and the phenolic oxygen in the salicylic acid moieties is removed too. These structural changes lowered the possibility that the compound 1 and 2 interfere with the free radical action of Aβ 1-40 compared with Chrysamine G and Congo Red. The compound 1 and 2 might coat the surface of Aβ 1-40 fibril and alter the surface of Aβ fibril. This surface modification might prevent the interaction between cell-surface receptor and Aβ fibril or complement proteins and Aβ fibril which could result in cell death. 14

In summary, the amide derivatives of Chrysamine G show high affinity for the Aβ fibril and protect human astrocyte cells against Aβ 1-40 fibril induced toxicity

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References


Figure 2. The protective effect of Aβ 1-40 fibril in astrocyte cells.