Abstract of Doctoral Dissertation

Degree requested: Doctor of Life Science　　Applicant's name: Hadya Virupaksha Deepak

Title of Doctoral Dissertation

Dual inhibitors of amyloid beta aggregation and sphingomyelin synthase from natural resources
アミロイドβ凝集とスフィンゴミエリン合成酵素の二重阻害を示す天然資源化合物に関する研究

Amyloid aggregation and misfolding of proteins are commonly considered as the basis for multiple neuro degenerative diseases such as Alzheimer's diseases (AD), Parkinson disease, prion disease and Huntington disease .AD is common form of dementia and its clinical manifestation characterized by progressive memory loss, visuospatial dysfunction, disorientation, behavioral turbulence, cognitive dysfunction and imbalances in lifestyle of elderly people particularly over 65-year-old. Drug design is the inventive process of finding new medications based on the knowledge of the biological target. The notion of 'one molecule – one target – one disease' has been a prevalent paradigm in pharmaceutical industry. The main idea of this approach is the identification of a single protein target whose inhibition leads to a successful treatment of the examined disease.

Modulation of the activity of a specific enzyme or receptor by a small molecule is one of the cornerstones of modern target-based drug discovery. However, small molecules frequently bind to multiple target molecules, influencing both drug efficacy and safety. Although the one-drug-hits-one target approach was dominant in the post-genomic era, it is regarded as inadequate to address complex diseases. The multitarget approach has become a fruitful area of drug discovery, particularly in developing medicines to treat major complex diseases. For example, natural products such as genistein, curcumin, and tashinone have been reported to inhibit amyloid β (Aβ) and human islet amylin (hIAPP) associated with type 2 diabetes. Another compound, miltirone, isolated from *Salvia miltiorrhiza*, was shown to be a dual inhibitor of P-glycoprotein and cell growth in doxorubicin-resistant HepG2 cells.

In this thesis, the author reports successful isolation of daurichromenic acid (1), as a potent dual inhibitor for sphingomyelin synthase (SMS) and amyloid beta (Aβ) aggregation (Figure 1).

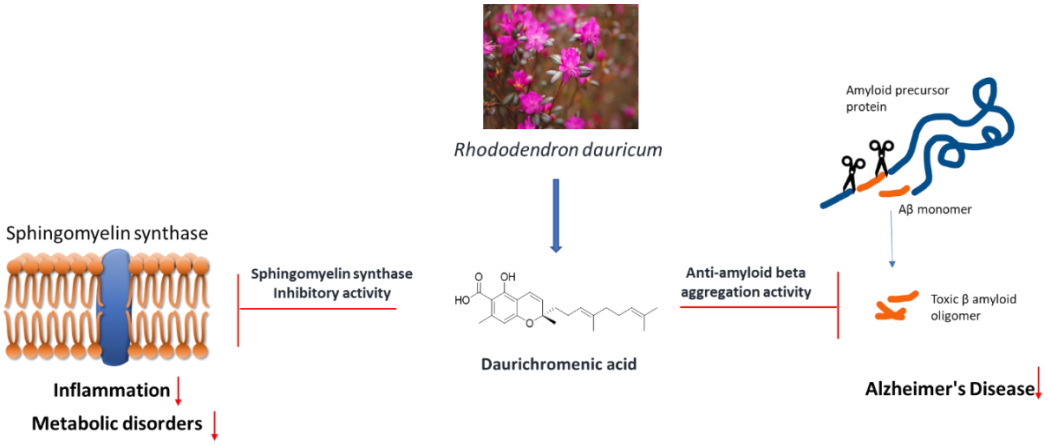

Figure 1. Dual inhibitor for SMS and Aβ aggregation inhibition from *Rhododendron dauricum*

To determine the biological properties of DCA and its derivatives and whether they are promising candidate drugs, the ability of these compounds to inhibit SMS activity was analyzed, as was the structure–activity relationship of these compounds in cell-based SMS assays. Furthermore, the effects of DCAs and natural SMS inhibitors on Aβ aggregation were assessed by microtiter-scale high-throughput screening (MSHTS) assays.

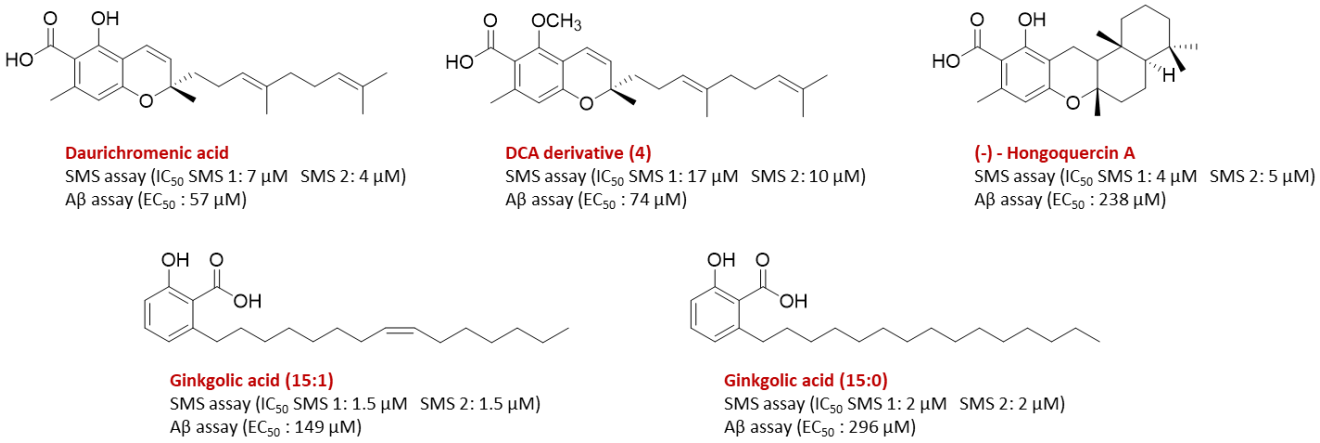

Figure 2. Dual inhibitors of SMS and Aβ aggregation

This study shows DCA, a terpenoid from the leaves of *Rhododendron dauricum*, is a natural inhibitor of sphingomyelin synthase. Few of its derivatives, including compounds 4, and (-) hongoquercin A, also showed good inhibitory activity against SMS. In addition, similar three compounds were found to inhibit $A\beta_{42}$ aggregation (Figure 2). Compounds with SMS inhibition properties against cell-lysate assays and the fluorescent substrate C6-NBD (4-nitrobenzo-2-oxa-1,3-diazole)-Cer share a carboxylic acid moiety which is also related to anti-aggregative properties against amyloid beta protein. MSHTS assays were used to evaluate amyloid beta aggregation activity using DCA and their derivatives. DCA (1) and compound 4 and (-) hongoquercin A with the presence of a carboxylic acid group tend to inhibit both SMS and Aβ aggregation inhibition. Best of our knowledge, this is the first report on daurichromenic acid and derivatives of DCA, compound 4 and (-) hongoquercin A as a dual inhibitor for sphingomyelin synthase and amyloid beta aggregation.