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Structure–Activity Relationship Study of Asiatic Acid Derivatives Against Beta Amyloid (Aβ)-induced Neurotoxicity

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Abstract—8 Semi-synthetic derivatives of asiatic acid were prepared and their protective effect against Aβ-induced neurotoxicity was evaluated. Among them, asiatic acid (**2**), and **4**, **16** showed 97, 92 and 87% of protective effect, respectively. © 2000 Elsevier Science Ltd. All rights reserved.

Dementia of Alzheimer's type in the elderly has become the main social problem. Recently it has been reported that the most important pathological hallmark of Alzheimer's disease (AD) is deposition of senile plaques in the brain.[1] The senile plaque consists of diverse molecules but the major component is beta amyloid (Aβ) protein which is concentrated in the plaque core.[2–4] Based on these results, the abnormal overproduction of Aβ has been proposed as a cause of AD.[5,6]

Centella asiatica is one of the herbal plants used in different continents by diverse ancient culture and tribal groups.[7] Historically, the extract has been used as a wound healing agent,[8] and brain tonics for the mentally retarded.[9] The extract has three different triterpenoid ingredients; asiaticoside (**1**), asiatic acid (**2**), and madecassic acid (**3**) (Fig. 1).[10,11] In 1992, the extract has been patented as a dementia treating agent and cognitive enhancer.[12] In this paper, the primary structure activity relationship (SAR) study of asiatic acid against Aβ-induced neurotoxicity were reported.

Structurally, asiatic acid (**2**) has three kinds of functional groups: three hydroxy groups at C(2), C(3) and C(23); an olefine group at C(12); a carboxylic acid group at C(28). Among these functional groups, the unusual triol and C(28)-carboxylic acid were modified and the protective effect against Aβ-induced neurotoxicity was evaluated.

Eight compounds were prepared from asiatic acid (**2**) which could be easily obtained from the titrated extract of *Centella asiatica*. We adapted the known procedure for the synthesis of **3**,[13] **4**,[13] **5**[14] and **6**.[15] C(2)-Ketoasiatic acid (**9**) was prepared from **6** in 3 steps. The direct oxidation of **6** in the presence of C(28)-CO_2H gave **9** in poor yield, but the corresponding ester (**7**) could be oxidized successfully. The treatment of **6** with ethoxymethyl chloride in basic condition gave **7**. The oxidation of **7** by using pyridinium chlorochromate followed by simultaneous deprotection of acetonide and ethoxymethyl ester in acidic condition gave **9**. C(2)-O-Acetylasiatic acid (**13**) was prepared from **7** by acetylation using acetic anhydride in basic condition and the following deprotection of acetonide and ethoxymethyl ester. In case of octyloxymethyl ester of asiatic acid (**11**), the octyloxymethyl ester formation from **6** by using octyloxymethylchloride followed by deprotection of acetonide in acidic condition gave **11** (Scheme 1).[16] C(2)-Deoxyasiatic acid (**16**) and C(2),C(3),C(23)-trideoxyasiatic acid (**19**) were prepared from **7** and **5**,

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respectively as shown in Scheme 2.[16] The treatment of **7** with CS_2, CH_3I in basic condition gave the xantate (**14**). The reduction of **14** with *n*-Bu_3SnH, followed by deprotection of acetonide gave **16**. Compound **19** could be obtained by similar method. Compound **5** was converted to trixantate (**17**) by addition of excess CS_2 and CH_3I in basic condition. The reduction of **17**, followed by hydrolysis with lithium bromide in collidine gave **19**.

In order to determine the lead compound, the in vitro protective effect against Aβ-induced neurotoxicity for the above asiatic acid derivatives (**4**–**6**, **9**, **11**, **13**, **16**, **19**)

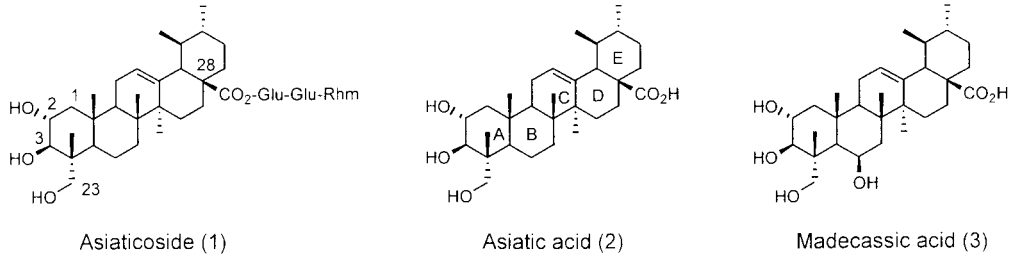
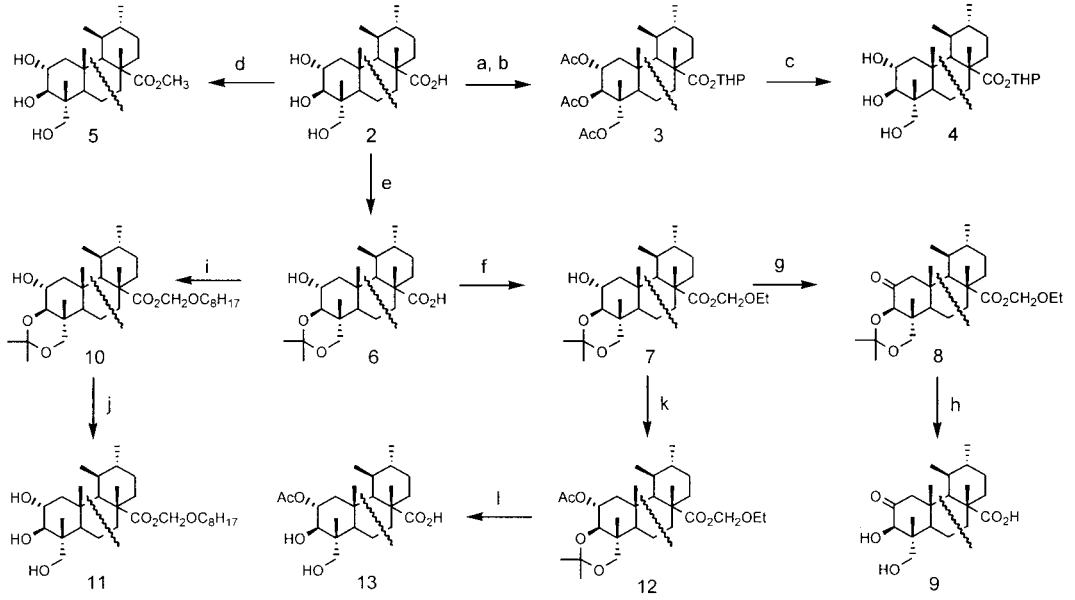
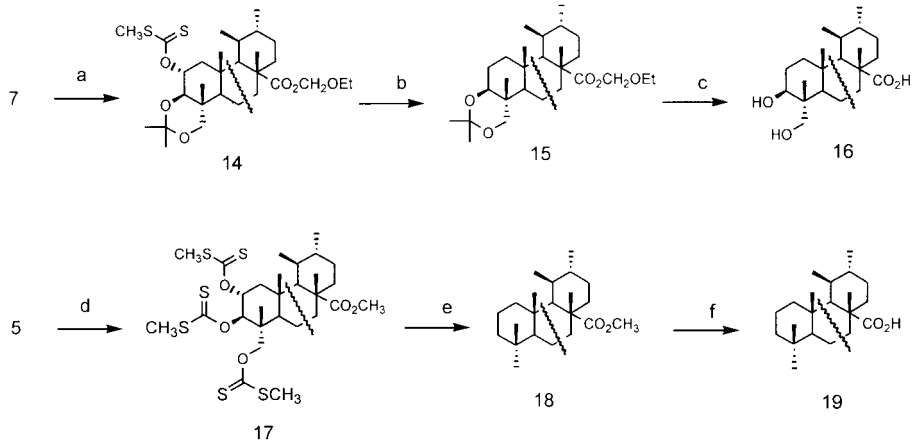


Figure 1.



Scheme 1. Conditions and reagents: (a) DHP, PTSA (cat.), THF, 0 °C, 1 h, 90%, (b) Ac_2O, Et_3N, CH_2Cl_2, rt, 3 h, 90%, (c) K_2CO_3, EtOH, rt, 2 h, 85%, (d) excess CH_2N_2, 0 °C, 1 h, 100%, (e) 2,2-dimethoxy propane, PTSA (cat.), DMF, rt, 5 h, 95%, (f) $EtOCH_2Cl$, $(i\text{-}Pr)_2NEt$, CH_2Cl_2, rt, 1 h, 95%, (g) PCC, CH_2Cl_2, rt, 4 h, 82%, (h) 6 N-HCl (cat.), CH_3OH, H_2O, rt, 2 h, 85%, (i) $C_8H_{17}OCH_2Cl$, $(i\text{-}Pr)_2Net$, CH_2Cl_2, rt, 1 h, 90%, (j) 1 N-HCl (cat.), CH_3OH, H_2O, 0 °C, 0.5 h, 85%, (k) Ac_2O, Et_3N, CH_2Cl_2, rt, 3 h, 85%, (l) 6 N-HCl (cat.), CH_3OH, H_2O, rt, 2 h, 85%.



Scheme 2. Conditions and reagents: (a) CS_2, CH_3I, NaH, THF, rt, 2 h, 77%, (b) *n*-Bu_3SnH, toluene, reflux, 2 h, 85%, (c) 6 N-HCl (cat.), CH_3OH, H_2O, rt, 2 h, 85%, (d) CS_2, CH_3I, NaH, THF, rt, 2 h, 64%, (e) *n*-Bu_3SnH, toluene, reflux, 2 h, 82%, (f) LiBr, collidine, reflux, 3 h, 75%.

Table 1. In vitro protective effect against Aβ-induced neurotoxicity for asiatic acid derivatives[17]

Compound	Protective activity (%)	Compound	Protective activity (%)
1	24	**9**	50
2	97	**11**	22
3	56	**13**	22
4	92	**16**	87
5	58	**19**	40
6	66		

along asiaticoside (**1**), asiatic acid (**2**), and madecassic acid (**3**) were investigated (Table 1). Inspection of Table 1 revealed several patterns in structure versus activity. First of all, the free C(28)-CO_2H derivative (**2**, 97%) showed higher protective activity than methyl, octyloxymethyl, and glycosyl ester derivatives (**5**, 58%; **11**, 22%; **1**, 24%, respectively). But the tetrahydropyranyl ester derivative (**4,** 92%) showed comparable activity with **2** (97**%**). The dramatic difference between **4** and **11** which both have similar alkyloxymethyl ester group might be due to the hydrolysis of **4** inside the neuronal cell. Secondly, the triol group is very important for biological activity, especially C(3)-OH and C(23)-OH. The C(2)-O-acetyl derivative (**13**, 22%) and C(2)-keto derivative (**9**, 50%) showed decreased activity versus **2** (97%), but the C(2)-deoxy derivative (**16**, 87%) showed comparable activity. This result suggested that the C(2)-OH is not quite critical but there is spatial constraint in the binding process. In case of C(3)-OH and C(23)-OH, the protection with C(3),C(23)-acetonide (**6**, 66%) reduced activity compared with **2** (97%). Deoxygenation of C(3)-OH and C(23)-OH of **16** reduced the activity by 50% (**16**, 87%; **19**, 40%). The additional hydroxy group at C(5) in madecassic acid (**3**, 56%) decreased activity. These cumulating results indicate that hydrogen bond interactions might play an important role in binding process with receptor in neuronal cell. In conclusion, SAR study was performed with semi-synthetic derivatives of asiatic acid. Among them, **2** (97%), **4** (92%) and **16** (87%) showed high protective activity against in vitro Aβ-induced neurotoxicity. This finding gives us valuable information to develop new drug for the treatment against Alzheimer's disease. Based on the in vitro result, the further detailed SAR study and in vivo study are currently being investigated.

Acknowledgements

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16. All new compounds gave satisfactory spectroscopic data consistent with the proposed structures.
17. The general assay method was as the following: after the pretreatment of asiatic acid derivatives (1 μM) to B103 cells for 1 h at 37 °C, cell death was induced by addition of $A\beta_{25-35}$ (50 μM) to the culture for 24 h at 37 °C. MTT assay was employed to quantitify cell survival. The degree of cell survival was measured based on the absorbance at O.D. 570–630 nm, using plate reader.