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Synthesis and neuroprotective effects of serofendic acid analogues

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Abstract—Analogues of serofendic acid were prepared and their protective effects against L-glutamate (Glu)-induced neurotoxicity were examined using primary cultures of rat cortical neurons. Some analogues exhibited similar neuroprotective activity to that of serofendic acid.
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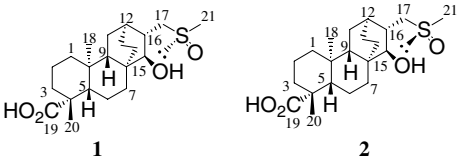
We have previously reported the isolation of two novel neuroprotective substances, serofendic acid A (**1**) and B (**2**), from lipophilic extracts of fetal calf serum, and confirmation of their structures by total synthesis (Fig. 1).[1,2] These compounds exhibited potent protective action against neurotoxicity induced by L-glutamate (Glu), which has been postulated to play important roles in the pathophysiology of many neurological diseases. Although the mechanism of the neuroprotective effect is unknown, we speculate that the serofendic acids interact with functional molecules involved in NO-mediated Glu-induced neurotoxicity.[1] Both the sulfur-containing atisane-type diterpenoid structure and the neuroprotective activity are of great interest. This article describes the synthesis and activity of a series of seorfendic acid analogues.

The synthesis of the analogues is outlined in Schemes 1 and 2. Compounds **5**, **7**, **10**, **13**, and **20**, intermediates in the total synthesis of serofendic acid,[2] were used to prepare the analogues. Oxidation of an epimeric mixture of serofendic acid (ca. A:B = 1:2) (**3**) using Dess–Martin periodinane[3] gave the C15 ketone analogue **4**. The sulfide **5** was oxidized with peracetic acid to afford the sulfone analogue **6**. Mesylation of the allyl alcohol **7**, followed by, thiomethylation, gave **8**. The methyl ester group of **8** was hydrolyzed with NaSMe, followed by oxidation of the sulfide group with Davis's oxaziridine[4] to give the C15-C16 dehydrated analogue **9**. Hydroboration of **10** gave the alcohol **11** as a C16 epimeric mixture (ca. 1:1), which was converted to the analogue **12** in a manner similar to that used for the synthesis of **9**.

The C19 methyl ester analogue **14** was prepared through tosylation of **13**, followed by thiomethylation and oxidation of the sulfide group. The C19 methyl analogue **17** was also prepared from **13**. Di-methoxymethylation of **13** followed by reduction with $LiAlH_4$ afforded **15**. The C19 alcohol group of **15** was deoxygenated by reaction with 1,1′-thiocarbonyldiimidazole, followed by reduction with tri-*n*-butyltin hydride.[5] The *O*-methoxymethyl groups were removed under acidic conditions to furnish the diol **16**, which was converted to **17** in a manner similar to that used for the synthesis of **14**. The 19-hydroxymethyl analogue **19** was prepared through reduction of **5** with $LiAlH_4$, followed by oxidation of the sulfide group. The stereoisomer of serofendic acid **22** was prepared from the diol **20** in a manner similar to that described for the synthesis of serofendic acid.[2]



1 **2**

Figure 1. Structures of serofendic acid A (**1**) and B (**2**).

Keywords: Serofendic acid; Neuroprotective; Glutamate; Atisane.
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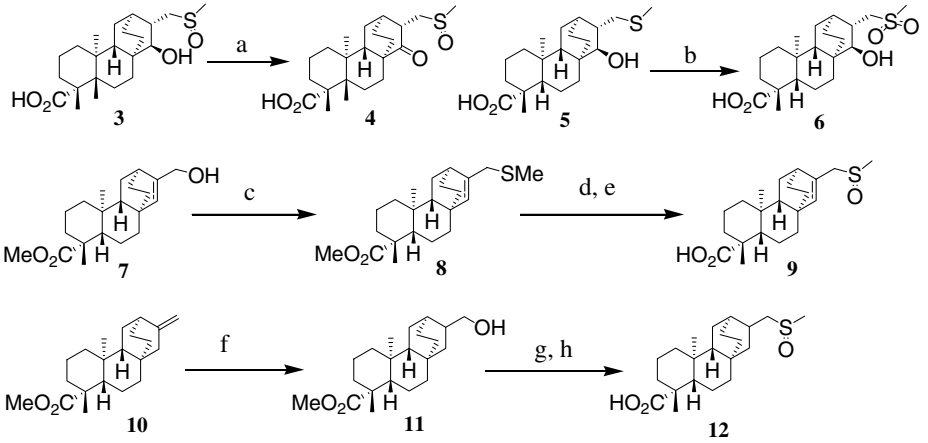
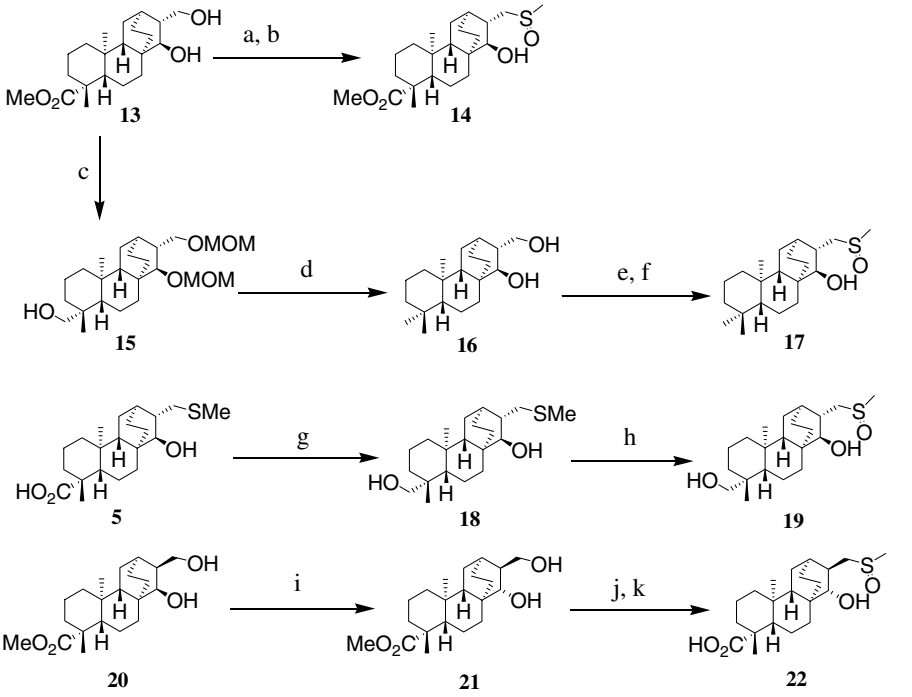


Scheme 1. Synthesis of analogues. Reagents and conditions: (a) Dess–Martin periodinane, CH_2Cl_2, 0 °C, 4 h, 51%; (b) H_2O_2, AcOH, 50 °C, 1 h, 37%; (c) i—MsCl, TEA, CH_2Cl_2, rt, 5 h, ii—NaSMe, DMF, 40 °C, 2 h, 46%; (d) NaSMe, HMPA, 80 °C, 54 h, 40%; (e) Davis's oxaziridine (2-benzenesulfonyl-3-phenyloxazirine), $CHCl_3$, 0 °C, 2 h, quant; (f) i—BH_3-THF, rt, 13 h ii—H_2O_2 aq, NaOH aq, rt, 1 h, 63%; (g) i—MsCl, TEA, CH_2Cl_2, ii—NaSMe, DMF, 60 °C, 5 h, 26%; (h) Davis's oxaziridine (2-benzenesulfonyl-3-phenyloxazirine), $CHCl_3$, 0 °C, 2 h, 84%.



Scheme 2. Synthesis of analogue. Reagents and conditions: (a) i—TsCl, DMAP, pyridine, rt, 13 h, ii—NaSMe, DMF, rt, 2 h, 27%; (b) Davis's oxaziridine (2-benzenesulfonyl-3-phenyloxazirine), $CHCl_3$, 0 °C, 1 h, 86%; (c) i—MOMCl, DIPEA, CH_2Cl_2, rt, 12 h, ii—$LiAlH_4$, THF, rt, 4 h, 98%; (d) i—thio-CDI, $ClCH_2$–CH_2Cl, rt, 19 h, ii—*n*-Bu_3SnH, AIBN, toluene, iii—7.5 N HCl aq, THF, rt, 3 h, 20%; (e) i—TsCl, DMAP, pyridine, rt, 23 h, ii—NaSMe, DMF, rt, 1 h, 60%; (f) Davis's oxaziridine (2-benzenesulfonyl-3-phenyloxazirine), $CHCl_3$, 0 °C, 1 h, 50%; (g) $LiAlH_4$, THF, reflux, 3 h, quant, (h) Davis's oxaziridine (2-benzenesulfonyl-3-phenyloxazirine), $CHCl_3$, 0 °C, 1 h, 63%; (i) i—$NaBrO_3$, $NaHSO_3$, CH_3CN, H_2O, rt, 2 h, 85%, ii—$NaB(OAc)_3H$, AcOH, CH_3CN, 0 °C, 80%; (j) i—TsCl, DMAP, pyridine, rt, 17 h, 63%, ii—NaSMe, HMPA, 80 °C, 40 h, 34%; (k) $NaIO_4$, MeOH, H_2O, 0 °C, 2 h, 67%.

Primary neuronal cultures were obtained from the cerebral cortex of fetal rats (17–19 days of gestation) and neurotoxicity was assessed by means of the Trypan blue exclusion method.[6] Exposure of rat cortical cultures to 100 μM Glu for 24 h markedly reduced the cell viability. We observed the neuroprotective activity of synthetic analogues (10 μM) when they were applied to the cultures for 1 h before and during the 24-h Glu (100 μM) exposure. Figure 2 shows the neuroprotective effect of serofendic acid epimeric mixture (**3**). Application of MK-801 (1 μM), a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist that binds to the phencyclidine binding site, completely blocked the Glu-induced neurotoxicity. We have previously reported that serofendic acid has no effect on NMDA-, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-, and kainate-evoked currents.[1] These findings indicate that the serofendic acid blocks Glu

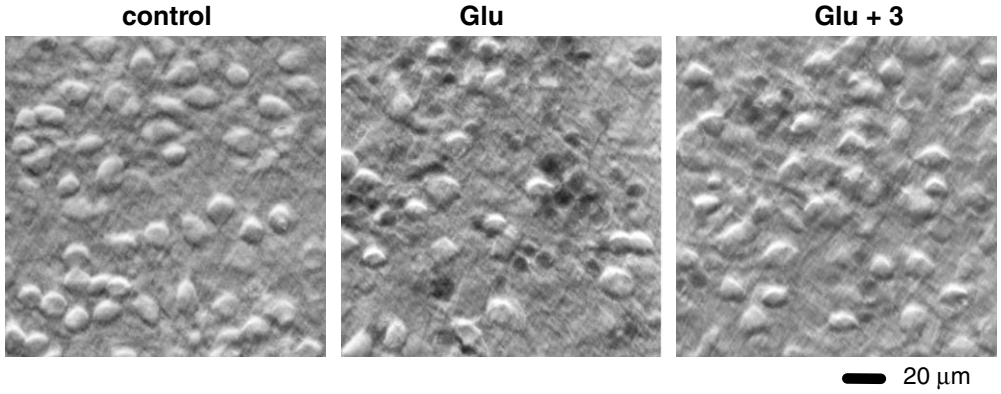


Figure 2. Hoffman modulation contrast photomicrographs showing the effects of serofendic acid (**3**). Cultures were treated with 100 μM Glu for 24 h. Serofendic acid **3** (10 μM) was applied to the culture medium from 1 h before and during the 24-h of Glu treatment.

neurotoxicity without affecting the function of glutamate receptor channels. To normalize the variation in the viability of cultures, the degree of protection induced by the test compounds was calculated using the following equation: protection (%) = ((C − B)/(A − B)) × 100,[7] where A is the viability of control cultures, B is the viability of the cultures treated with Glu, and C is the viability of the cultures treated with Glu and the test compound. An example of the protection afforded by serofendic acid (**3**) is shown in Figure 3.

In the previous study, compounds **1** and **2** (serofendic acid A and B), as well as the sulfoxide epimeric mixture **3**, potently attenuated Glu neurotoxicity to similar extents.[8] Therefore, the analogues having the sulfoxide group were tested as epimeric mixtures. The C15 ketone analogue **4** exhibited a significant neuroprotective effect (Table 1). The sulfoxide moiety at C17 position is an unique feature of serofendic acid, so we compared the effects of sulfoxide, sulfone, and sulfide moieties. The sulfide **5** showed potent activity, but the sulfone **6** was less active. The analogues **9** and **12** lacked activity, indicating that the oxygen functionality at the C15 position may be important for neuroprotective effect. The C15-C16 di-*epi* analogue **22** was as potent as serofendic acid, suggesting that the absolute stereochemistry at C15 and C16 may be of minor importance.

The neuroprotective activities of analogues at the C19 position are shown in Table 2. The methyl ester ana-

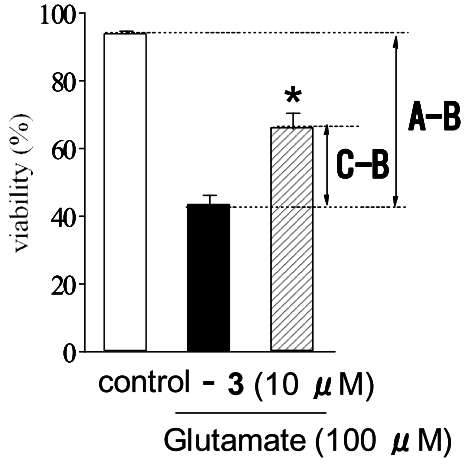
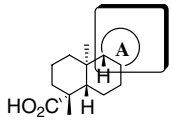
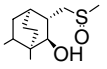
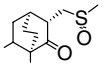
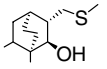
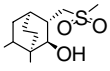
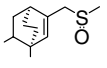
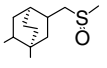
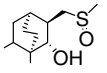


Figure 3. Protective effect of serofendic acid (**3**) against Glu-induced neurotoxicity. Cultures were treated with 100 μM Glu for 24 h. Serofendic acid **3** (10 μM) was applied to the culture medium from 1 h before and during 24 h of Glu treatment. $n = 5$. Data were expressed as means ± SEM. The statistical significance of difference between groups was determined by one-way analysis of variance (ANOVA) followed by Dunnett's two-tailed test. Statistical significance was defined as probability value of less than 5%.

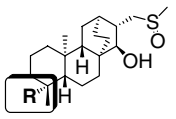
Table 1. Neuroprotective effects of serofendic acid analogues



Compound	A	Protection (%)	Statistical significance
MK-801 (1μM)	—	80.7	*
Serofendic acid (**3**)		45.3	*
4		41.5	*
5		39.8	*
6		20.0	n.s.
9		15.6	n.s.
12		18.1	n.s.
22		33.7	*

n.s., no statistical significance.
*$p < 0.05$ vs glutamate-treated group.

Table 2. Neuroprotective effects of serofendic acid analogues



Compound	R	Protection (%)	Statistical significance
Serofendic acid (3)	CO_2H	45.3	*
14	CO_2Me	20.5	*
17	Me	13.1	n.s.
19	CO_2OH	7.79	n.s.

n.s., no statistical significance.
*$p < 0.05$ vs glutamate-treated group.

logue **14** exhibited weak activity. Both methyl analogue **17** and hydroxymethyl analogue **19** exhibited loss of activity. These results may indicate that the carboxylic acid moiety at this position plays an important role in neuroprotective activity.

In summary, our synthetic route to serofendic acid permitted the synthesis of various analogues, some of which exhibited neuroprotective effects similar to that of serofendic acid. The C15 oxygen functionality and the C19 carboxylic acid moiety appear to be important for the activity. Further study will be needed to establish the structure–activity relationship of the pharmacophore in detail.

References and notes

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