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Discovery of Synthetic Methoxy-substituted 4-Phenylbutyric Acid Derivatives as Chemical Chaperones

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In this study, we evaluated the chemical chaperone activity of synthetic 4-phenylbutyric acid (4-PBA) derivatives. These derivatives have a methoxy group at the benzene ring and/or longer or shorter fatty acid portions. Several 4-PBA derivatives demonstrated higher antiaggregation activity than 4-PBA. Moreover, 4-(4-methoxyphenyl)butanoic acid (**7b**) showed protective effects against endoplasmic reticulum stress-induced neuronal cell death.

4-Phenylbutyric acid (4-PBA), **1** (Figure 1), is a terminal aromatic-substituted fatty acid and a well-known chemical chaperone. It has been used to treat disorders of the urea cycle. We reported that 4-PBA protects against cerebral ischemic injury and endoplasmic reticulum (ER) stress-induced neuronal death, demonstrates chemical chaperone activity, and prevents the aggregation of reduced α (alpha)-lactalbumin (r-LA) with denatured bovine serum albumin (BSA).[1,2] Moreover, we reported that the chemical chaperone activity and protective effects of ER stress-induced neuronal death are dependent on the number of carbon atoms bound to the benzene ring.[3]

4-PBA has remarkable potential as a novel therapeutic agent for type-2 diabetes and familial hypercholesterolemia.[4] Furthermore, 4-PBA exerts significant neuroprotective effects in mouse models of Parkinson's disease (PD) and Alzheimer's disease (AD).[5–7] Although these effects are valuable, a high dose of 4-PBA is required. Therefore, reducing the dose of 4-PBA when using it as a drug is an important limitation that should be overcome. Conversely, the chemical chaperone activity of 4-PBA derivatives has not been reported. Hence, we evaluated certain synthetic 4-PBA derivatives for their chemical chaperone activity against denatured proteins.

The synthetic routes to 4-PBA derivatives are illustrated in Scheme 1. General experiments are shown in the Supporting Information. We had thought that chemical chaperone activity is dependent on the benzene ring, because in a previous paper, butyrate had showed the very weak chemical chaperone activity.[3] Therefore, we focused on the benzene ring substituted with fatty acids, not on the fatty acid in 4-PBA.

First, we examined the effects of compounds as chemical chaperones in in vitro aggregation of α-LA with BSA. Experimental details are shown in the Supporting Information. As a representative of the compounds, we have shown the

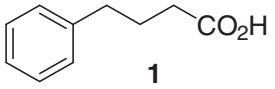

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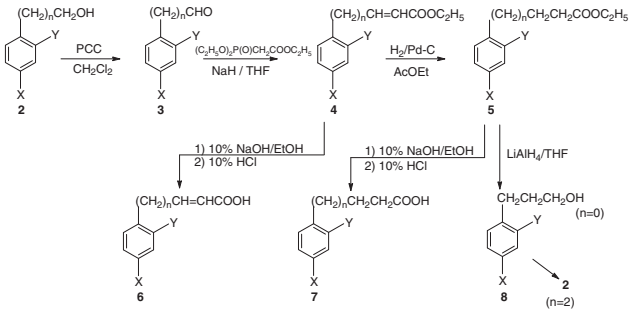
Figure 1. Structure of 4-PBA.



6a: n= 0, X= OCH_3, Y= H (64, 2) **7a**: n= 0, X= OCH_3, Y= H (92, 3)
6b: n= 1, X= OCH_3, Y= H (27 ,3) **7b**: n= 1, X= OCH_3, Y= H (37, 4)
6c: n= 2, X= OCH_3, Y= H (51, 6) **7c**: n= 2, X= OCH_3, Y= H (35, 7)
6d: n= 0, X= H, Y= OCH_3 (99, 2) **7d**: n= 0, X= H, Y= OCH_3 (60, 3)
6e: n= 1, X= H, Y= OCH_3 (38, 3) **7e**: n= 1, X= H, Y= OCH_3 (28, 4)
6f : n= 2, X= H, Y= OCH_3 (36, 6) **7f** : n= 2, X= H, Y= OCH_3 (33, 7)
overall yields (%, steps)

Scheme 1. Synthetic scheme of 4-PBA derivatives.

inhibitory effect of **6a** on the protein aggregation (Figure 2 upper). Moreover, the chemical chaperone activities of 0.3, 1, and 3 mM 4-PBA derivatives were compared with that of 3 mM 4-PBA (Figure 2 lower). All derivatives inhibited the aggregation of denatured BSA and r-LA in a concentration-dependent manner. In particular, six derivatives **6b**, **6e**, **7a**, **7b**, **7c**, and **7f** suppressed aggregation more strongly than 4-PBA at the same concentration. This result showed that 4-PBA, available as a seed compound, may be used against neurodegenerative diseases if a minor structural change is induced in it.

With respect to the structure–activity relationship, inhibitory effects increased if the methoxy group was substituted in the *para* position (fourth/sixth) of the benzene ring. However, for derivatives having a methoxy-substituted benzene ring, the relationship between chemical chaperone activity and the number of carbon atoms bound to the benzene ring was not investigated. Chemical chaperone activity was dependent on the

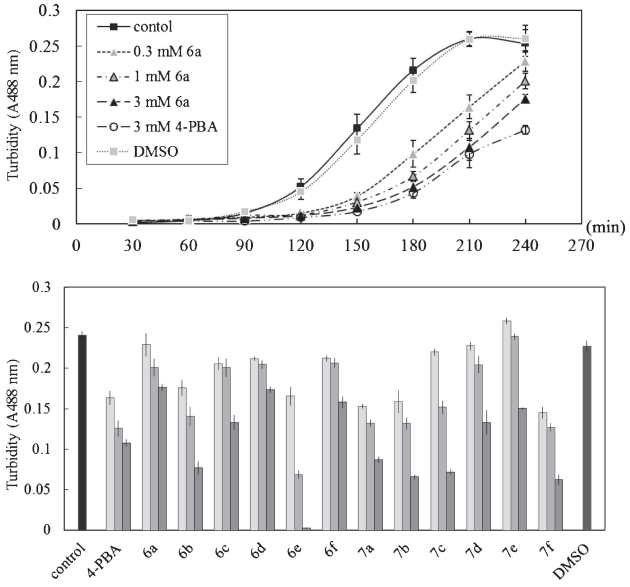


Figure 2. In vitro inhibitory effects of 4-PBA derivatives on r-LA aggregation.

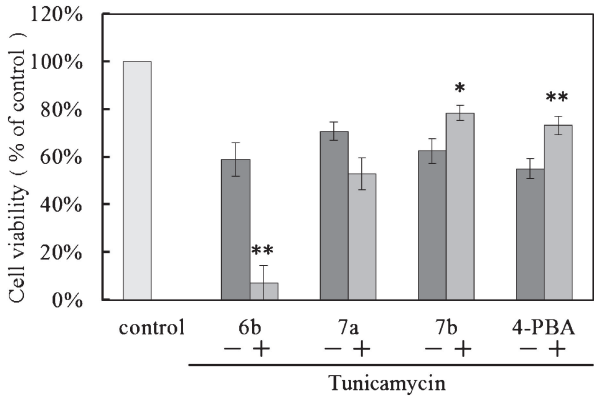


Figure 3. Protective effects of compounds against ER stress-induced cell death in human neuroblastoma SH-SY5Y cells (three independent experiments in duplicate) ($^{*}p < 0.05$, $^{**}p < 0.01$: compared with vehicle control, Student's *t*-test).

number of carbon chains bound to the benzene ring (i.e., higher number of carbon chains, higher tendency), which was demonstrated in our previous study[3] but not observed in the present study. The double bond in the parts of the fatty acid in the derivative was not necessarily required for the inhibition of aggregation (**7a**–**7f** vs. **6a**–**6f**). The *para*-position of methoxy group in the benzene ring was effective in aggregation inhibition (**7a**–**7c** vs. **7d**–**7f**). In olefins, the position of the methoxy group was not effective (**6a**–**6c** vs. **6d**–**6f**).

Next, we investigated whether compounds **6b**, **7a**, and **7b** possessed protective effects against neuronal death induced by ER stress with tunicamycin (Figure 3). Experimental details are shown in the Supporting Information. Recently, ER stress has been shown to participate in neurodegenerative diseases, particularly AD and PD. We reported that 4-PBA protected ER stress-induced neuronal death.[1–3] **7b** showed effects that were equal to or better than that of 4-PBA.[3] **6e** was the most effective compound for aggregation inhibition. However, **6e** (1 mM) significantly increased activated caspase-3-positive cells (The ratio of activated caspase-3-positive cells to total cells: control $3.857 \pm 0.684\%$, **6e** $16.1^{*} \pm 1.897\%$, $^{*}p < 0.05$ compared with vehicle control, Dunnett's test). This result suggested that **6e** might possess the apoptosis-inducing activity.

In conclusion, we showed that minor structural changes in 4-PBA enhanced the inhibition of protein aggregation. Moreover, 4-(4-methoxyphenyl)butanoic acid (**7b**), which is a synthetic methoxy-substituted 4-PBA derivative, showed protective effects against ER stress-induced neuronal cell death (Figure 3). These results raise the possibility that **7b** is more versatile than 4-PBA. Moreover, **7b** may reduce the dose required for efficacy in a mouse model of neurodegenerative diseases. 4-PBA has been patented to be a therapeutic agent against AD in Japan.[8] However, further optimization is necessary for the safe use of 4-PBA as a therapeutic drug.[9]

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