【Advantageous effects】

The pyrrolidine derivative represented by Formula I, II, III, IV, or V, or a pharmaceutically acceptable salt thereof disclosed herein is highly effective in inhibiting aggregation of beta-amyloids and/or tau proteins and/or degrading the aggregates of beta-amyloids and/or tau proteins, and is thus highly useful for preventing and/or treating neurological diseases caused by aggregation and/or accumulation of beta-amyloids and/or tau proteins.

【Description of Drawings】

FIGS. 1A and 1B are diagrams identifying that TAU-1 and TAU-2 PROTAC compounds containing the compound according to the present invention exhibit degradation activity in three types of phosphorylated tau proteins. Specifically, FIG. 1A is an image obtained using ImageQuant LAS 4000 (GE healthcare, IL, USA) by western blotting with respect to the tau degradation activity of TAU-1 and TAU-2, and FIG. 1B shows the result of quantification of the image using Image J program.

FIG. 2 is a graph showing the binding affinity ($K_d$) of TAU-1 and TAU-2 PROTAC compounds containing the compound according to the present invention to a nonpathological WT tau protein and a pathological P301S tau protein.



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phosphorylated tau proteins, TAU-1 PROTAC exhibited more effective degradation activity in P-Tau S396, and TAU-2 PROTAC exhibited more effective degradation activity in P-Tau S202, T205, and S356. Accordingly, the compounds according to examples of the present invention have phosphorylated tau degradation activity and are particularly useful for the treatment of brain diseases attributable to phosphorylated tau proteins.

**Example 8. Determination of binding affinity ($K_d$) of tau PROTAC to WT tau or pathological tau**

The following experiment was performed to verify that the compound according to the present invention selectively degrades only phosphorylated tau.

In order to biophysically analyze selective binding of the tau PROTAC to P301S, which is known as a representative mutant of pathological tau, non-pathological (WT) tau and pathological (P301S) tau were cloned into pRSET-B vectors to obtain plasmids. Star pLysS cells were used to overexpress proteins and 1 mM IPTG was added to the medium at an $OD_{600}$ of 0.4 to 0.6. The intracellular proteins were extracted from the cells lysed in a lysis buffer through heat shock (95°C), and WT tau and P301S tau were obtained at a high purity (>95%) using cation exchange chromatography and size exclusion chromatography.



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Whether or not the obtained proteins bound to the tau PROTAC was determined through an SPR experiment. A CM5 sensor chip including dextran attached thereto was used, tau PROTAC was allowed to flow and the binding affinity between the protein and the compound was determined based on a response unit (RU). The binding affinity ($K_d$) to the protein was measured using concentration-dependent kinetic assay. The results of measurement of binding affinity between the tau PROTAC compound and non-pathological WT Tau or pathological P301S tau are shown in FIG. 2. As can be seen from FIG. 2, the tau PROTAC selectively binds to the pathological P301S, but hardly binds to nonpathological WT tau. Tau-1 PROTAC 1 and Tau-2 PROTAC compounds were found to have binding affinity to tau of 190.05 μM and 198.7 μM, respectively, which means that the compounds have very low or no binding affinity. On the other hand, Tau-1 PROTAC 1 and Tau-2 PROTAC compounds had binding affinity to the P301S tau of 42.05 μM and 18.74 μM, respectively. Therefore, the compounds according to the examples of the present invention are degrading only pathological tau without degrading nonpathological WT tau through selective binding to P301S tau, thus being useful for the treatment of brain diseases.



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