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(54) Title: INHIBITORS OF MEMAP SIN 2 AND USE THEREOF

(57) Abstract: Methods for the production of purified, catalytically active, recombinant memapsin 2 have been developed. The substrate and subsite specificity of the catalytically active enzyme have been determined by a method which determines the initial hydrolysis rate of the substrate by using MALDI-TOF/MS. Alternatively, the subsite specificity of memapsin can be determined by probing a library of inhibitors with memapsin 2 and subsequently detecting the bound memapsin 2 with an antibody raised to memapsin 2 and an alkaline phosphatase conjugated secondary antibody. The substrate and subsite specificity information was used to design substrate analogs of the natural memapsin 2 substrate that can inhibit the function of memapsin2. The substrate analogs are based on peptide sequences, shown to be related to the natural peptide substrates for memapsin 2. The substrate analogs contain at least one analog of an amide bond which is not capable of being cleaved by memapsin 2. Processes for the synthesis of substrate analogues including isoterres at the sites of the critical amino acid residues were developed and the more than seventy substrate analogues were synthesized, among which MMI-005, MMI-012, MMI-017, MMI-018, MMI-025, MMI-026, MMI-037, MMI-039, MMI-040, MMI-066, MMI-070, and MMI-071 have inhibition constants in the range of $1.4\text{--}61.4 \times 10^9$ M against recombinant pro-memapsin 2. These inhibitors are useful in diagnostics and for the treatment and/or prevention of Alzheimer's disease.

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