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RESEARCH ARTICLE

Antifungal dipeptides incorporating an inhibitor of homoserine dehydrogenase

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The antifungal activity of 5-hydroxy-4-oxo-L-norvaline (HONV), exhibited under conditions mimicking human serum, may be improved upon incorporation of this amino acid into a dipeptide structure. Several HONV-containing dipeptides inhibited growth of human pathogenic yeasts of the Candida genus in the RPMI-1640 medium, with minimal inhibitory concentration values in the 32 to 64 μg mL^{-1} range. This activity was not affected by multidrug resistance that is caused by overexpression of genes encoding drug efflux proteins. The mechanism of antifungal action of HONV dipeptides involved uptake by the oligopeptide transport system, subsequent intracellular cleavage by cytosolic peptidases, and inhibition of homoserine dehydrogenase by the released HONV. The relative transport rates determined the anticandidal activity of HONV dipeptides.

KEYWORDS

antifungal agents, dipeptides, homoserine dehydrogenase, oligopeptide uptake

1 | INTRODUCTION

Several inhibitors of enzymes catalyzing particular steps of amino acid biosynthesis pathways exhibit antifungal properties.[1] One of them is an antibiotic RI-331 (5-hydroxy-4-oxo-L-norvaline/2-amino-5-hydroxy-4-oxopentanoic acid, HONV), produced by *Streptomyces* spp.[2] This compound is active *in vitro* against several human pathogenic yeasts and the plant pathogen *Cladosporium fulvus* but has no effect against *Aspergillus* spp.[3-5] Furthermore, RI-331 was shown to be effective in the treatment of systemic murine candidiasis, being well tolerated in mice.[3,4] The molecular target of HONV is homoserine dehydrogenase, the key enzyme in the fungi-specific biosynthetic pathway of L-methionine, L-isoleucine, and L-threonine biosynthesis.[4-6] HONV acts as an enzyme-assisted suicide inhibitor of this enzyme.[7] Interestingly, this compound also modulates a glutathione-related drug resistance of tumor cells.[8]

Because homoserine dehydrogenase is a cytosolic enzyme, HONV must be internalized, ie, has to cross the cytoplasmic membrane to reach its target. The hydrophilic nature of its structure precludes fast passive diffusion, so the only possibility is an active transport by one of the amino acid permeases. These transporters, however, demonstrate strict substrate specificity, usually limited to proteinogenic amino acids. Therefore, the inefficient transport through the cytoplasmic membrane limits an antifungal activity of HONV. One of the possible approaches to solve this problem could be the application of the so-called Trojan horse strategy, ie, conjugation of an enzyme inhibitor with a molecular nanocarrier.[9] In the case of inhibitors of amino acid structure, facilitated transport is possible after their incorporation into oligopeptides, which are effectively taken up by oligopeptide permeases, demonstrating broad substrate specificity. Once internalized, such oligopeptides are cleaved by intracellular peptidases to release the inhibitor. Several examples of the successful application of this strategy for the construction of antimicrobials have been reported in the literature.[10-13] Herein, we present results of our studies on synthesis and biological properties of dipeptides incorporating HONV.

2 | RESULTS AND DISCUSSION

2.1 | Rationale for the construction of HONV-containing dipeptides

The amino acid inhibitor of homoserine dehydrogenase (HONV; Figure 1) was used for the construction of dipeptides as potential antifungal agents. The rationale for the design of HONV-containing oligopeptides was based on the previous findings concerning the optimization of structures of such compounds aimed at the maximization of uptake by oligopeptide transport systems, namely, (1) a preference