Fine tuning the substrate specificity of alcohol dehydrogenase from *Geotrichum candidum* by rational mutation

([1]*Department of Life Science and Technology, School of Life Science and Technology, Institute of Science Tokyo,* [2]*Department of Organic and Inorganic Chemistry, University of Alcalá*)
○Zhongyao Tang[1], Guillermo Germán Otárola Tejada[1,2], Afifa Ayu Koesoema[1], Tomoko Matsuda[1]

Keywords: Alcohol Dehydrogenase; Rational Mutation; Diaryl Ketone; Asymmetric Reduction; Medium-chain Dehydrogenase/Reductase

Chiral diaryl methanols are important building blocks in pharmaceutical molecules such as L-cloperastine (antitussive and antihistamine), (*S*)-neobenodine, and levocetirizine. Due to the mild reaction conditions, asymmetric reduction of diaryl ketones by alcohol dehydrogenases (ADHs) has attracted increasing attention as an environmentally friendly approach. The use of a novel ADH, *Geotrichum candidum* NBRC 4597 acetophenone reductase (*Gc*APRD), appears promising, as it has been successfully used in the reduction of various 2-benzoylpyridine analogs to produce the corresponding alcohols by rational mutations at residues 56 (in the large binding pocket) and 288 (in the small binding pocket)[1,2].

Driven by the success of benzoylpyridines reductions, in this study, we investigated the reduction of substituted benzophenones using two *Gc*APRD mutants, Trp288Ala and Phe56Ile/Trp288Ala. Benzophenones are more challenging than benzoylpyridines, due to the less electrophilicity of the carbonyl carbon, greater steric hindrance, and higher similarity between the two aryl groups[3-5].

Firstly, the reduction of 4'-chlorobenzophenone with *E. coli* whole-cell expressing the Phe56Ile/Trp288Ala mutant was conducted; the corresponding (*S*)-alcohol, the building block for L-cloperastine, was obtained with 80% *ee* (Fig.). With the successful reduction of 4'-chlorobenzophenone, the reduction of other substituted benzophenones and the reactions by Trp288Ala were also investigated, resulting in giving the corresponding (*S*)-alcohols in up to 86% *ee* (Fig.).

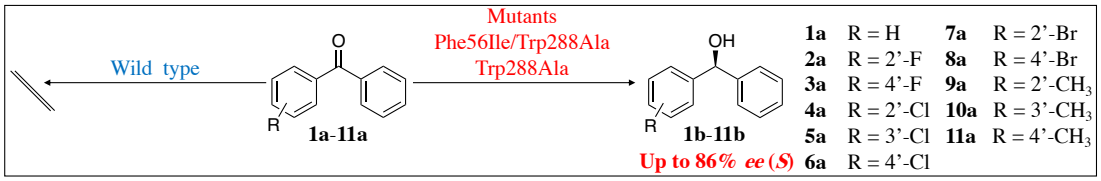

Fig. Asymmetric reduction of substituted benzophenones by *Gc*APRD mutants

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