Development of technology package for alcohol dehydrogenases (ADH) catalyzed synthesis of chiral alcohols

Project Lead: Prof. Pramod Wangikar

Specific problem being addressed: Phenol biotransformation-based processes have been attracting attention as greener alternatives to conventional synthesis. In the pharmaceutical industry, a number of chemical synthesis processes have been replaced with *chemo-enzymatic routes* with potential for many more processes to be substituted. The rising demand for enantio-pure intermediates in the fine chemicals and pharmaceutical industries makes biocatalysis an increasingly profitable alternative to conventional chemical catalysis. The current methods have several limitations. Our technology is expected to overcome the existing limitations and provide a successful commercial application that will require an enzyme that is highly active and stereoselective towards the substrate of choice, tolerates high substrate concentrations, is stable and amenable to recycling and is affordable. We aim to fill the current void, by developing chiral secondary alcohols of immense industrial importance that can be used as building blocks in the manufacture of various drugs such as antidepressants, antiviral and anti-Alzheimer's drugs.

Project Summary: Alcohol is a common functional group in organic chemistry, either as constituent of the final product or as "handle" for further synthetic transformation. Chiral alcohols play a key role as intermediates in synthesis of high value products of pharmaceutical importance. Alcohol dehydrogenase (ADH) catalyzed asymmetric reduction is a powerful approach to synthesize chiral alcohols.

Table 1. List of compounds for which ADH catalysed process will be developed in the proposed project.

Substrate	Product (Chiral intermediate)	Active pharmaceutical ingredient
Cholesterol lowering agents		
1. 4-chloro- 3-oxobutanoic acid methyl ester	(S)-4-chloro-3-hydroxybutanoic acid methyl ester	Atorvastatin
2. Ethyl-4-chloro acetoacetate	Ethyl (S)-4-chloro-3-hudroxybutyrate	Atorvastatin
3. 3,5-dioxo-6-(benzyloxy) hexanoic acid, ethyl ester	(3R,5S)-dihydroxy-6-(benzyloxy) hexanoic acid, ethyl ester	Atorvastatin and Rosuvastatin
Anti-anxiety / Antidepressent drug:		
4. 6-oxobuspirone/ 6-ketobuspirone	(S) 6-hydroxybuspirone/ (R) 6-hydroxybuspirone	Bispirone (Buspar)
5. 2, 3', 4'-trichloroacetophenone	2-chloro-1-(3,4-dichlorophenyl)ethanol	Sertraline
HIV protease inhibitor		
6. (1S)-[3-chloro- 2-oxo-1-(phenylmethyl)propyl] carbamic acid, 1,1-dimethylethyl ester	(1S,2R)-[3-chloro-2-hydroxy-1-(phenylmethyl) propyl]carbamic acid, 1,1-dimethylethyl ester	Atazanavir (acyclic aza-peptidomimetic)
Anti-Alzheimer's drug		
7. 2'-bromo- 4'-fluoroacetophenone	(S)-1-(2'-bromo-4'-fluorophenyl)ethanol	Anti- Alzheimer's drugs

The PI and the industry partner Hi Tech BioSciences Ltd., have been jointly working on ADH catalyzed biotransformation processes for over 3 years. We have significant leads in terms of i) novel enzyme that is active, stable and stereoselective, ii) high cell density process to produce recombinant ADH enzymes and iii) biotransformation process that affords favorable space time yield, solute to solvent ratio and cofactor TTN total

turnover number). We have demonstrated this for the conversion of ethyl 3-oxo-4-chlorobutanoate (COBE) to ethyl (S)-3-hydroxyl-4-chlorobutanoate [(S)-CHBE] at gram level.

Thus the present project has two objectives:
I: Optimization, validation and scale-up of biotransformation technology for the production of SDCHBE and demonstration at kilogram level
II: Enzyme engineering for the synthesis of other commercially important chiral alcohols as shown in Table 1 in the technical details section.

Impact of the innovation: The project deals with enzyme engineering for the synthesis of commercially important chiral alcohols, high cell density cultivation of E. coli culture to produce recombinant ADH enzyme and optimization, validation of the catalysis process. There are several challenges currently faced in the ADH enzyme study namely, i) High-cost of enzymes: This is partially due to the fact that certain companies enjoy monopoly in the enzyme business. ii) Substrate specificity and stereo-selectivity of enzyme: The wild-type enzyme may not have high activity or stereo-selectivity for the chosen substrate. iii) Industrial applications require solute to solvent ratio to be ~ 1:10 while literature reports that of ~ 1:100. iv) Recycling of the enzyme and co-factors is needed to achieve the desired co-factor and enzyme TTN. A successful commercial application therefore will require an enzyme that is highly active and stereoselective towards the substrate of choice, tolerates high substrate concentrations, is stable and amenable to recycling and is affordable. Our technology aims to fill this void, by developing chiral secondary alcohols are of immense industrial importance that can be used as building blocks in the manufacture of various drugs such as antidepressants, antiviral and anti-Alzheimer's drugs.