BIOTRANS
LA ROCHELLE 2023

N°840 / PC
TOPIC(s) : Enzyme discovery and engineering / Industrial biocatalysis

Biocatalysis in drug design: Engineered RedAm to access chiral building block with multiple stereocenters

AUTHORS

Arnau RUE / UNIVERSITY OF MANCHESTER, 131 PRINCESS ST, MANCHESTER
Martin HAYES / ASTRAZENECA, PEPPAREDSLEDEN 1, MÖLNDAL
Nicholas TURNER / UNIVERSITY OF MANCHESTER, 131 PRINCESS ST, MANCHESTER

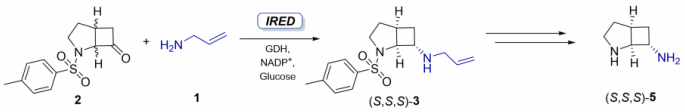
PURPOSE OF THE ABSTRACT

The pharmaceutical industry is constantly looking for innovative ways to access bioactive compounds with new structure-activity relationships (SAR), this has increased the demand for novel building blocks in drug design. A wide variety of these compounds allows to cover a big spectrum of different drug properties such as increased permeability, potency enhancement or different selectivity. Complex molecules are key to expand compound libraries, however chiral compounds with multiple stereocenters can be particularly difficult to access by traditional organic synthesis. Biocatalysis can be a powerful tool to cover this space since it can access new synthetic methodologies and it allows to control the stereoselectivity of the reaction. Moreover, its inherent ability to produce natural products derivates, which tend to naturally interact with protein drug targets, is highly desirable.

Herein, we report a biocatalytic process to access the desired enantiomer of a building for drug discovery, this molecule has multiple chiral centers and proved to be hardly accessible by organic synthesis. The first step aimed to identify active RedAms, however none of identified hits produced the desired enantiomer. Therefore, IR-09 was engineered following a semi-rational mutagenesis approach, which was designed around the principle that diastereoselectivity and enantioselectivity of the enzyme are based on the orientation of the ligand in the active site. A crystal structure of IR-09 was obtained and target residues for site directed mutagenesis were identified. Finally, variants W204A and W204S were able to access the desired enantiomer (S,S,S). These results suggested that residue W2404 was key to switch selectivity, hence site saturation mutagenesis was performed. New variants which could also produce the (S,S,S)-enantiomer were identified. For example, W204R exhibited the highest selectivity yielding 45% e.r.. which is very close to the theoretical maximum e.r. (50%), since the starting material is a racemic mixture.

Evolution of this enzyme not only allowed to switch the stereoselectivity but also to incorporate stereospecificity for the substrate. This is the case of variant W204G, which has preference for the (S,S) starting material, thus performing selective reductive amination and concomitant kinetic resolution of the substrate. Furthermore, this biocatalytic step can be incorporated into a chemoenzymatic cascade to enable the production of the primary amine. Finally, the evolved RedAm proved to retain high levels of conversion (>90%) on preparative scale, therefore being an interesting backbone for further evolution in case the drug progresses to process development.

FIGURES



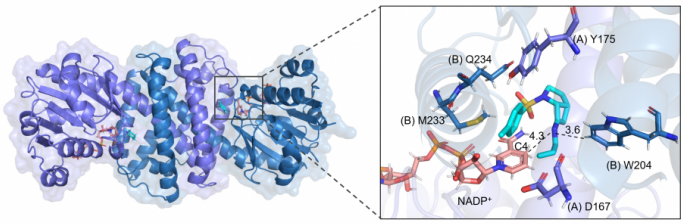


FIGURE 1

Synthetic route to key building block

Enzymatic reductive amination of ketone 2 with allylamine (1) to provide (S,S,S)-3, which can be easily converted to key component (S,S,S)-5.

FIGURE 2

IR-09 crystal structure and active site

IR-09 crystal structure and closer look to the active site with ligand and targeted residues for mutagenesis.

KEYWORDS

Biocatalysis | RedAm | Semi-rational mutagenesis | Drug design

BIBLIOGRAPHY

Devine, P. N.; Howard, R. M.; Kumar, R.; Thompson, M. P.; Truppo, M. D.; Turner, N. J. Extending the Application of Biocatalysis to Meet the Challenges of Drug Development. Nat. Rev. Chem. 2018, 2 (12), 409-421. https://biotrans2023.livescience.io/#

Fryszkowska, A.; Devine, P. N. Biocatalysis in Drug Discovery and Development. Curr. Opin. Chem. Biol. 2020, 55, 151-160.

Aleku, G. A.; France, S. P.; Man, H.; Mangas-Sanchez, J.; Montgomery, S. L.; Sharma, M.; Leipold, F.; Hussain, S.; Grogan, G.; Turner, N. J. A Reductive Aminase from Aspergillus Oryzae. Nat. Chem. 2017, 9 (10), 961-969.