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Breakthrough in the challenging P450-catalyzed chemoenzymatic synthesis of C14-functionalized steroids

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Intensive past research has demonstrated that cytochrome P450 monooxygenases are involved in the biosynthesis of numerous natural products and in human metabolism of various pharmaceuticals [1]. This inspired synthetic organic and pharmaceutical chemists to test them as catalysts in the oxidative hydroxylation of steroids. Unfortunately, wildtype P450s generally proved to show low levels of regio- and diastereoselectivity as well as suboptimal activity. In order to solve these (and other) challenging problems associated with P450s [2], efficient methods in semi-rational directed evolution were developed [3]. Rather than utilizing random mutagenesis of the type epPCR, or DNA shuffling, such focused mutagenesis techniques as Combinatorial Active-site Saturation Test (CAST) and Iterative Saturation Mutagenesis (ISM) were conceived and implemented [4], and subsequently applied to P450-catalyzed oxidative hydroxylation of steroids. For example, planned C7- and C16-selectivities were achieved [5,6]. Today, improved versions of CAST/ISM are considered to have rational rather than just semi-rational character [3].

Zhou, Qu and co-workers have taken a significant step forward in selective P450-catalyzed synthesis of C14-functionalized steroids recently [7]. This difficult problem was solved in two phases, the first involving a clever strategy based on discovering the proper P450 monooxygenase and performing mutagenesis using alanine scanning, which allowed for the identification of hot-spots in the enzyme. The second phase was designed to ensure regio-and stereoselectivity in favor of the steroidal position C14, made possible by applying CAST/ISM. A number of structurally different steroids reacted regio- and stereoselectively (Figure 1, top). A theoretical analysis revealed the origin of selectivity (Figure 1, bottom). In addition to oxidative hydroxylation, chemoenzymatic sequences led to variously functionalized steroids, which are also valuable for potential pharmaceutical applications [7].

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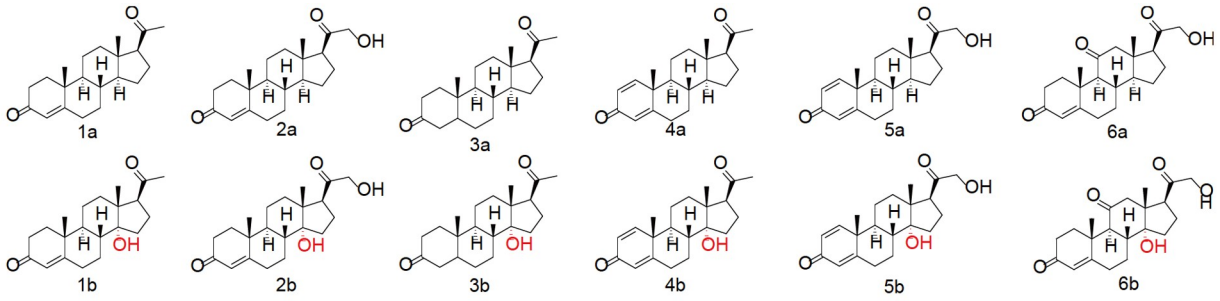

Enz.	WT	I111A	V124A	M115K	I111L	I111L-M115K	I111-V124W
Sub.	C/S (%)	C/S (%)	C/S (%)	C/S (%)	C/S (%)	C/S (%)	C/S (%)
1a	48.5/77.3	62.1/83.7	49.3/76.4	46.3/74.8	67.4/85.2	62.3/87.1	75.3/92.2
2a	65.3/73.4	71.4/84.7	70.4/80.1	68.3/88.2	70.3/90.7	71.4/92.6	72.5/94.4
3a	61.5/85.2	67.7/88.4	65.5/86.3	67.4/90.5	57.7/70.4	63.5/77.2	71.7/90.2
4a	56.2/69.0	37.4/45.1	52.5/70.8	73.5/85.3	60.7/77.1	75.5/91.8	72.4/86.3
5a	45.3/57.4	15.2/20.5	44.7/58.4	76.5/92.2	57.5/69.5	70.7/86.4	77.8/90.3
6a	70.3/92.1	65.5/90.3	74.6/94.2	63.6/86.5	72.5/90.2	76.793.2	78.3/94.5

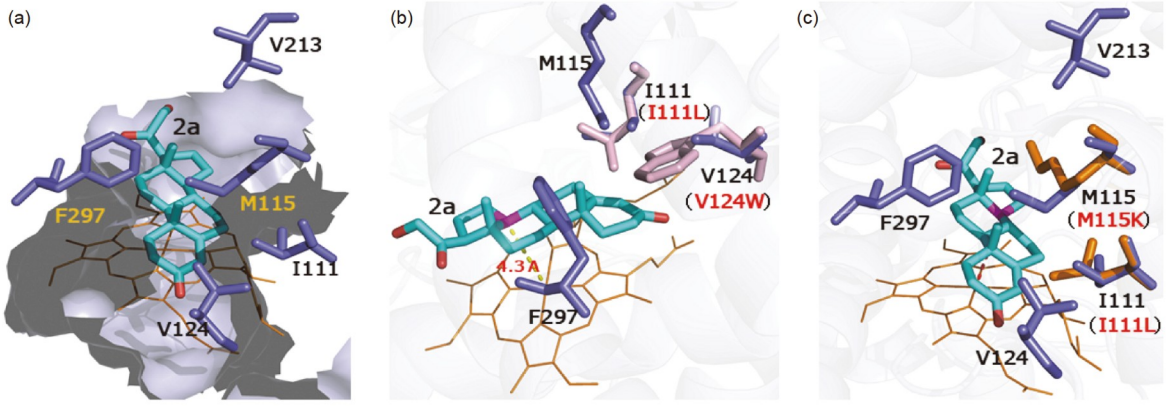

Figure 1 P450-catalyzed synthesis of C14α-OH steroids. Top: structurally different steroids and their respective product C14α alcohols. Below: in silico model of CYP14A with substrate **2a**. (a) The binding cavity of CYP14A. Substrate **2a**, haem and the binding cavity are colored cyan, brown, and light blue, respectively. The four critical residues, as well as V213, are labelled by the corresponding amino acids. (b) Comparison of WT enzyme and IIIIL-V124W. The mutated residues and C14 of the substrate are colored pink. (c) Comparison of the WT enzyme and I111L-M115K. The mutated residues and position C14 of the substrate are colored brown and pink, respectively. Adapted with permission from Ref. [7], copyright by The Author(s) (2023).

Conflict of interest The authors declare no conflict of interest.

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