Effect of mutations of *Thermoanaerobacter ethanolicus* secondary alcohol dehydrogenase at tryptophan-110 on enantioselectivity of reduction of phenyl–ring containing ketones

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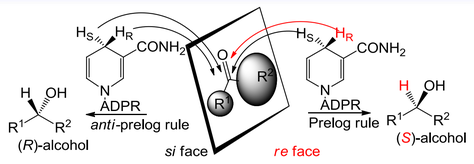
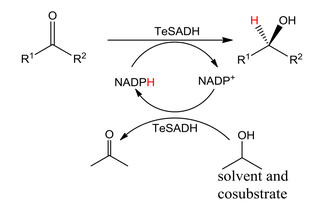
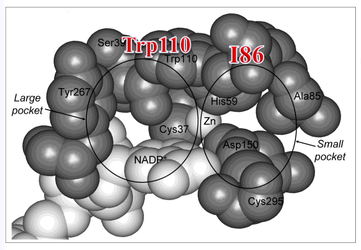
Abstract

- The asymmetric reduction of selected phenyl-ring-containing ketones by various mutants of Thermoanaerobacter ethanolicus secondary alcohol dehydrogenase (TeSADH) was studied using single and dual site mutagenesis.
- For substituted 2-tetralones we noticed that the changing in the position of the substituent on the aromatic ring also has a great impact on the binding affinity and maximum catalytic rate, as reflected by the kinetic parameters V_{max} and K_m.
- We also explored the regio- and enantioselective reduction of diketones using W110A/I86A TeSADH.
- The expansion of both small and large pockets of TeSADH in the mutant W110A/I86A not only accommodates the substrates of single mutants W110A and I86A within the active site, but also expands the substrate scope to ketones bearing two sterically demanding groups (bulky-bulky ketones); which are not substrates for TeSADH single mutants.

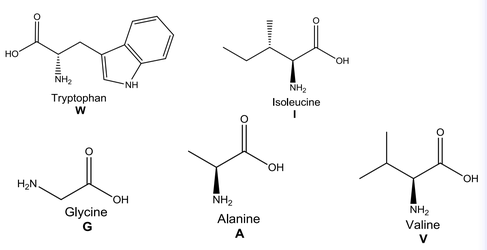
Introduction





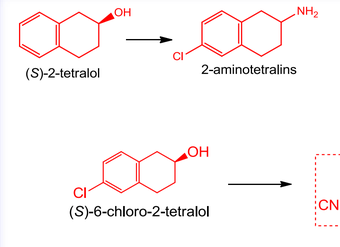


The active site of TeSADH

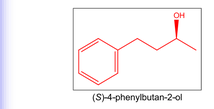


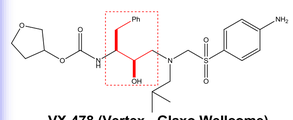
W110G
W110A
W110V
W110A / I86A

Significance of Study

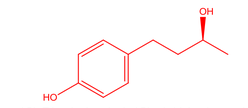


- Treats nervous disorders
- Active to dopamine & serotonin receptors
- Synthon in *anti*-myocardial ischemia
- Precursor for MK-0499 (treats ventricular arrhythmias) [1].



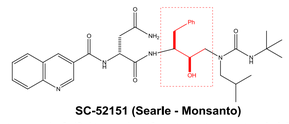


VX-478 (Vertex - Glaxo Wellcome)



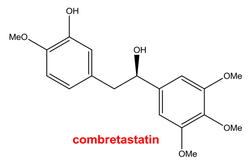
- ✓ hepato-protective
- ✓ melanin-inhibitor

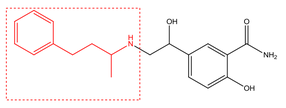
(skin lighting cosmetics) [3].



SC-52151 (Searle - Monsanto)

[protease inhibitors in the treatment of AIDS] [2].





Labetalol
(treats high blood pressure)

- ✓ 1,2-diarylethanols use in the preparation of combretastatin & analogs as *anti*-cancer agents [4].

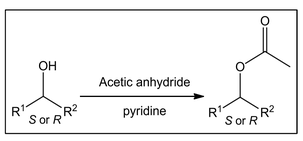
Materials and Methods

procedure for reaction

- Substrate (0.0191 mmol)
- TeSADH (W110G, W110A, W110V, W110A/I86A)
- Tris-HCl buffer solution (50 mM, pH 8.0)
- $NADP^+$ (1 mg)
- 50 °C, 24 hr.
- extraction with ethyl acetate

stereochemical outcome

- ✓ Polrimetry : to measure the optical rotation of alcohol product
- ✓ GC equipped with a chiral column: to determine the *ee* of the corresponding acetate esters
- ✓ HPLC equipped with a chiral column

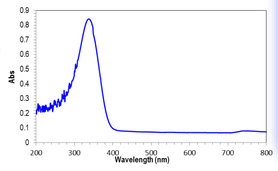


Chemical identity

- ✓ ^1H and ^{13}C NMR
- ✓ IR
- ✓ EI MS

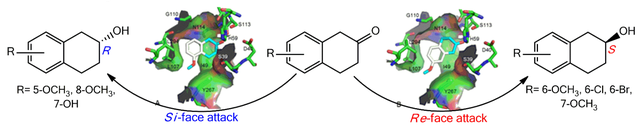
Enzyme kinetics

- Substrate concentration (0.001-10 mM)
- Tris-HCl buffer solutions (pH 6.5, 50 mM)
- CH_3CN (10%)
- NADPH (0.25 mM)
- 340 nm
- 50°C



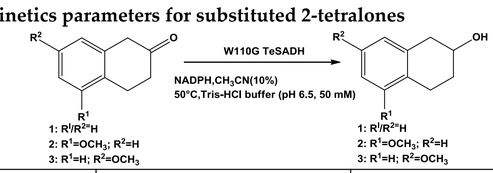
Results and Discussion

➢ The asymmetric reduction of substituted 2-tetralones



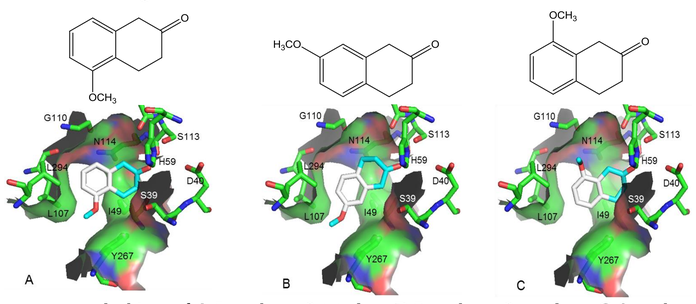
Substrate	Product	Mutant TeSADH	Conv. (%)	ee (%)
		W110G	>99	69
		W110A	>99	71
		W110V	5	91
		W110A/I86A	12	87
		W110G	>99	61
		W110A	99	87
		W110V	96	71
		W110A/I86A	>99	95
		W110G	>99	>99
		W110A	99	>99
		W110V	46	>99
		W110A/I86A	>99	>99
		W110G	>99	>99
		W110A	99	>99
		W110V	67	>99
		W110A/I86A	94	>99
		W110G	>99	>99
		W110A	99	>99
		W110V	86	>99
		W110A/I86A	95	>99

➢ Kinetics parameters for substituted 2-tetralones



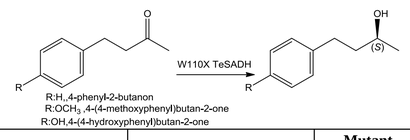
Ketone	W110G TeSADH		W110A TeSADH	
	K_m (mM)	V_{max} (mM.min^{-1})	K_m (mM)	V_{max} (mM.min^{-1})
	0.0184 ± 0.000824	0.0748 ± 0.00335	0.00580 ± 0.00029	0.0589 ± 0.0048
	0.0231 ± 0.0011	0.0666 ± 0.00316	0.0106 ± 0.000528	0.0695 ± 0.0039
	0.0452 ± 0.00156	0.0743 ± 0.00257	0.0122 ± 0.000611	0.0545 ± 0.00218

➢ Docking results for substituted 2-tetralones



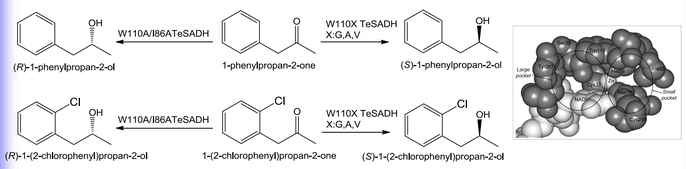
Lowest energy dockings of **A**:5-methoxy-2-tetralone,**B**:7-methoxy-2-tetralone, **C:**8-methoxy-2-tetralone in the catalytic site of W110G TbADH. The substrate is viewed from the face occupied by NADP in the TeSADH crystal structure.

➢ The asymmetric reduction of substituted 4-phenyl-2-butanone



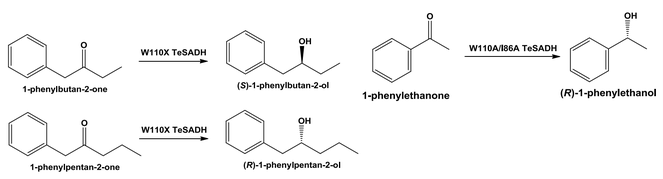
Substrate	Product	Mutant TeSADH	Conv. (%)	ee (%)
		W110G	>99	96
		W110A	99	99
		W110V	95	>99
		W110A/I86A	91	98
		W110G	93	20
		W110A	98	60
		W110V	94	79
		W110A/I86A	40	84
		W110G	95	99
		W110A	97	>99
		W110V	92	94
		W110A/I86A	80	>99

➢ The asymmetric reduction of substituted phenyl acetone



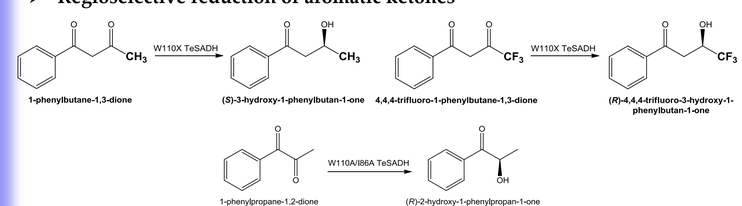
Substrate	Configuration of Product	Mutant TeSADH	Conv. (%)	ee (%)
	(S)	W110G	>99	79
	(S)	W110A	95	84
	(S)	W110V	>99	>99
	(R)	W110A/I86A	>99	80
	(S)	W110G	13	89
	(S)	W110A	84	98
	(S)	W110V	99	>99
	(R)	W110A/I86A	>99	99

➢ The asymmetric reduction of other aromatic ketones



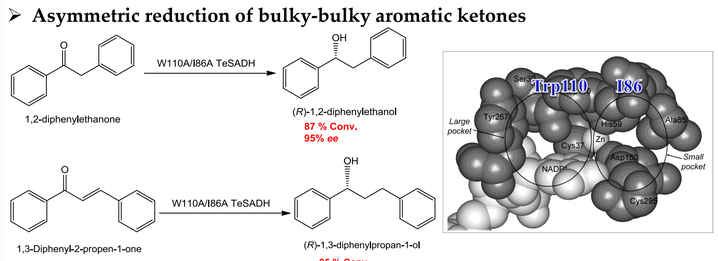
Substrate	Products	Mutant TeSADH	Conv. (%)	ee (%)
		W110G	36	93
		W110A	98	92
		W110V	98	>99
		W110A/I86A	72	71
		W110G	nr	n.d
		W110A	3	n.d
		W110V	32	92
		W110A/I86A	95	98
		W110G	nr	nr
		W110A	nr	nr
		W110V	nr	nr
		I86A	79	79
		W110A/I86A	46	46

➢ Regioselective reduction of aromatic ketones



Substrate	Product	Mutant TeSADH	Conv. (%)	ee (%)
		W110G	92	>99
		W110A	99	>99
		W110V	89	>99
		W110A/I86A	92	92
		W110G	3	n.d
		W110A	8	n.d
		W110V	>99	>99
		W110A/I86A	2	n.d
		W110G	nr	nr
		W110A	nr	nr
		W110V	nr	nr
		W110A/I86A	>99	>99

➢ Asymmetric reduction of bulky-bulky aromatic ketones



Conclusion

- W110A/I86A TeSADH expanded the substrate scope for TeSADH.
- Various mutants of TeSADH reduced diketones in high regioselectivity and enantioselectivity.
- A reversed enantiopreference for few substrates was observed when W110A/I86A TeSADH was used as a catalyst.
- The interactions between the ketone and the residues of the active site of enzyme determine the mode of substrate binding, and thus the enzyme's steroselectivity.

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References **[1]**. Reddy, J. *et al. Ferment. Bioeng*. **1996**, *81* (4), 304–309. **[2]**. Johnson, J. *et al. Pharmacotherapy*. **2000**, *20*, 622-628. **[3]**. Yuasa, Y. *et al. Synth. Commun*. **2003**, *33* (9), 1469–1475. **[4]**. Johnson, J. A . *et al. Pharmacotherapy*. **2000**, *20*, 622-628. **[5]**. Bornscheuer, U. T. *et al. Nature*. **2012**, *485*, 185-194. **[6]**. Prelog, V. *Pure Appl. Chem*. **1964**, *9*, 119-130 **[7]**. Kroutil, W. *et al. Curr. Opin. Chem. Biol*. **2004**, *8*, 120-126. **[8]**. Musa, M. M *et al. J. Org. Chem*. **2007**, *72*, 30-34. **[9]**. Musa, M. M. *et al. ChemCatChem*. **2009**, *1*, 89-93. **[10]**. Ziegelmann-Fjeld, K. I. *et al. Protein Eng. Des*. **2007**, *20*, 47-55.