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CH-activating oxidative hydroxylation of 1-tetralones and related compounds with high regio- and stereoselectivity

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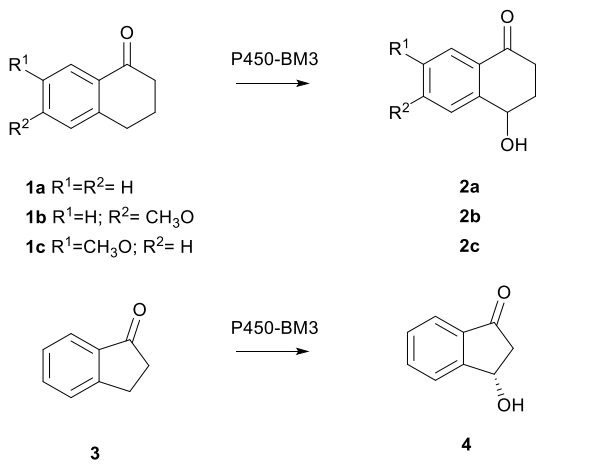
Mutants of P450-BM3 evolved by directed evolution are excellent catalysts in the CH-activating oxidative hydroxylation of 1-tetralone derivatives and of indanone, with unusually high regio- and enantioselectivity being observed. Similar results were achieved in the oxidative hydroxylation of tetralin and indane. The products are useful building blocks in the synthesis of a number of biologically active compounds.

4-Hydroxy-1-tetralones of the type **2a-c** are valuable constituents and/or building blocks of a number of biologically active natural products and pharmaceuticals. Examples include glucosides from *Juglans mandshurica*[1] containing (*S*)-**2a**, which have been used in Chinese folk medicine to treat cancer, dermatosis and pain, as well as the fresh pericarps of *Juglans sigillata*[2] also employed in folk medicine in Asia and Europe.[3] More recent examples are 8MAPK inhibitors as anti-inflammatory agents in the treatment of respiratory diseases.[4] Few catalytic methods for the asymmetric synthesis of this class of compounds have been developed.[5] We envisioned a one-step access by P450-catalysed CH-activating oxidative hydroxylation[6] of readily available 1-tetralones **1a-c** (Scheme 1), the challenge being the control of regio- and enantioselectivity.[7] The present study also includes 1-indanone (**3**) and the saturated analogs tetralin and indane as substrates. The possible use of chiral synthetic catalysts for this type of selective transformation has not been reported to date.[8]



1a R^1=R^2= H
1b R^1=H; R^2= CH_3O
1c R^1=CH_3O; R^2= H

2a
2b
2c

3

4

Scheme 1 P450-catalysed oxidative hydroxylation of 1-tetralones (**1a-c**) and 1-indanone (**3**).

In earlier studies we utilized P450-BM3 (CYP102A1) from *Bacillus megaterium*[6,9] as the catalyst in the oxidative hydroxylation of steroids[10a] and of small molecules such as cyclohexene-1-carboxylic acid ester[10b] and methylcyclohexane,[10c] regio- and stereoselectivity being controlled by directed evolution[11] based on saturation mutagenesis at sites aligning the binding pocket.[12] In the present study we first tested WT P450-BM3 and 25 previously evolved mutants[10c] in the hydroxylation of the model compound **1a**. Whereas WT led to essentially complete regioselectivity in favor of the desired (*S*)-4-hydroxy-1-tetralone (**2a**), enantioselectivity proved to be poor (33% ee). In contrast, several mutants showed excellent regio- and enantioselectivity (Table 1, entries 2-5). All of them are characterized by point mutations at residue A328, which shows that this position is a "hot spot" as noted in other studies.[6] Indeed, in the case of the other two substrates **1b-c**, the best mutants likewise show amino acid substitutions at position 328. Surprisingly, in the reaction of 6-methoxy-1-tetralone (**1b**) reversal of enantioselectivity in favour of

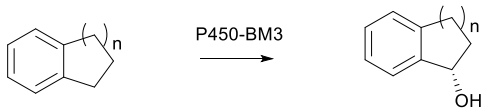
(*R*)-**2b** was observed (95% ee), while regioselectivity reaches only 48% (Table 1, entry 7). Therefore, saturation mutagenesis was performed at residue A328 (using NNK degeneration), which resulted in the identification of a notably improved variant A328P showing enhanced regioselectivity in favor of the 4-position while maintaining high enantioselectivity (94% ee) (Table 1, entry 8). This library was then screened in the attempt to identify a catalyst for the hydroxylation of substrate **1c** which is superior to WT (essentially racemic **2c**; Table 1, entry 9). The best variant proved to be A328I which shows enhanced regioselectivity compared with A328F, but at slight expense of enantioselectivity (Table 1, entries 10-11). In the case of 1-indanone (**3**), WT P450-BM3 resulted in 98% regioselectivity but moderate enantioselectivity (76% ee in favor of (*S*)-**4**), while variant A328R constitutes a nearly perfect catalyst in terms of overall selectivity. It is interesting to note that the *Pseudomonas* sp. strain 9816/11 expressing naphthalene dioxygenase (NDO) leads to the enantiomeric product (*R*)-**4**.[13] Thus, in this particular case NDO and P450-BM3 variant A328R are complementary biocatalysts. The performance of NDO in the oxidation of 1-tetralone derivatives has not been reported to date.

Table 1 P450-BM3 catalysed oxidative hydroxylation of ketones **1a-c** and **3** with formation of (*S*)-**2a-c** and **4**.[a]

Entry	Substrate	P450-BM3	Product	%-Regio.	%-Enantio.	TOF[b] [min^{-1}]	%-Conv.[b,c]
1	**1a**	WT	**2a**	99	33, (*S*)	1.9	86
2	**1a**	A328F	**2a**	98	99, (*S*)	3.8	>99
3	**1a**	A328K	**2a**	99	96, (*S*)	-[d]	56
4	**1a**	A328R	**2a**	99	88, (*S*)	-	59
5	**1a**	A328Y	**2a**	99	97, (*S*)	-	39
6	**1b**	WT	**2b**	97	82, (*R*)[e]	2.2	86
7	**1b**	A328F	**2b**	48	95, (*R*)	1.3	64
8	**1b**	A328P	**2b**	85	94, (*R*)	-	71
c	**1c**	WT	**2c**	91	1, (*S*)[e]	6.2	88
10	**1c**	A328F	**2c**	50	99, (*S*)	3.0	75
11	**1c**	A328I	**2c**	84	86, (*S*)	-	92
12	**3**	WT	**4**	98	76, (*S*)	0.9	47
13	**3**	A328F	**4**	98	89, (*S*)	1.9	96
14	**3**	A328K	**4**	95	93, (*S*)	-	37
15	**3**	A328R	**4**	98	96, (*S*)	0.2	45

[a] Values were obtained by averaging at least three independent experiments performed with resting cells at 5 mM. [b] TOF and conversions were calculated for WT and the best mutants. [c] Conversion calculated after 20 h. [d] Not determined. [e] Absolute configuration assigned after NMR analysis of derivatized alcohols **2b-c** with Mosher chloride and also comparison of the optical rotation signs of **2b-c** with the optical rotation sign of **2a**.

Finally we tested some of the best mutants as catalysts in the oxidative hydroxylation of indane (**5a**) and tetralin (**5b**). In the former case excellent regio- and enantioselectivity is possible using variants A328K or A328Y (Table 2, entries 8, 10). High regioselectivity was also achieved in the C-H activating hydroxylation of indane, but maximum enantioselectivity did not exceed 83% ee. Noyori-type Ru-catalyzed reduction of indanone (**3**) constitutes the superior strategy in this case.[14] Hydroxylated products **6a-b** or its derivatives are of great biological importance.[15]



5a n= 1 **6a**
5b n= 2 **6b**

Scheme 2 CH-activating oxidative hydroxylation of indane (**5a**) and tetralin (**5b**).

Table 2 P450-BM3 catalysed oxidative hydroxylation of tetralin (**5a**) and indane (**5b**).[a]

Entry	Substrate	P450-BM3	Product	%-Regio.	%-Enantio.	TOF[b] [min^{-1}]	%-Conv.[b,c]
1	**5a**	WT	**6a**	>95	<1, (*S*)	14.9	>99
2	**5a**	A328F	**6a**	>95	83, (*S*)	6.1	>99[d]
3	**5a**	A328K	**6a**	>95	68, (*S*)	-	83
4	**5a**	A328R	**6a**	>95	78, (*S*)	-	59
5	**5a**	A328Y	**6a**	90	61, (*S*)	-	67
6	**5b**	WT	**6b**	92	56, (*S*)	4.9	>98
7	**5b**	A328F	**6b**	90	99, (*S*)	13.9	>99[d]
8	**5b**	A328K	**6b**	97	98, (*S*)	-	>99
9	**5b**	A328R	**6b**	98	98, (*S*)	-	>99
10	**5b**	A328Y	**6b**	97	97, (*S*)	-	>99

[a] Values were obtained by averaging at least three independent experiments performed with resting cells at 5 mM. [b] TOF and conversions were calculated for WT and the best mutants. [c] Conversion calculated after 20 h. [d] Reactions reached total conversion after 1 h.

In an attempt to characterize and understand the origin of selectivity of some of the variants, kinetic studies and docking/molecular dynamics (MD) experiments were performed (see SI). The accepted mechanism of P450-catalysed oxidative hydroxylation involves H-atom abstraction by the catalytically active heme-Fe^{V}=O species (Compound I), with intermediate formation of an alkyl radical, followed by rapid C-O bond formation.[5,8] Using 1-tetralone (**1a**) as the model substrate, a docking calculation was first

performed on the WT enzyme (details in SI). Previous computational studies of P450-catalysed hydroxylation of several different substrates have revealed that the ideal angle of approach of the hydrogen atom undergoing abstraction should be at approximately 130° to the Fe^{V}=O fragment.[16] Several docking poses of 1-tetralone (**1a**) were observed, but only a single pose satisfied this criterion. In that pose, the pro-(*S*) hydrogen at C4 favors abstraction, consistent with our experimental findings. In this pose, the substrate is positioned within a hydrophobic pocket above the heme, and in contact with F87. In order to investigate the conformational dynamics of 1-tetralone in the active site of the WT enzyme, two independent unrestrained MD simulations were performed on this docked structure (details in SI). The simulations reveal significant tumbling of the substrate around the hydrophobic pocket and no hydrogen bonds are observed between the substrate carbonyl oxygen and the active site residues. The latter finding is consistent with the similar selectivity patterns observed for substrates **1a** and **5b**, as well as **3** and **5a**. Substrate **1a** rarely gets close enough to the Fe^{V}=O moiety for reaction to occur, however such events do take place at multiple instances during the timescale of the simulations (48 ns) and the pro-(*S*) hydrogen at C4 is the favored atom to undergo abstraction (Figure 1).

In order to understand the unexpected switch in enantioselectivity when subjecting 6-methoxy-1-tetralone (**1b**) to hydroxylation, we performed analogous docking experiments. In this case the highest-ranking docking pose was observed where the pro-4(*R*) hydrogen is in a reactive position. In this position, the tetralone is flipped over (relative to the position of substrate **1a**) such that the phenyl group (and methoxy substituent) points towards the I-helix. An additional docking pose was found in which the phenyl group points away from the I-helix and the pro-4(*S*) hydrogen was closest to the heme, however the calculated binding affinity was less favorable for this position (by 0.5 kcal/mol). 7-methoxytetralone (**1c**) was also docked into the WT crystal structure. Two binding poses were observed of equivalent binding affinity, corresponding to abstraction of the pro-4(*S*) and pro-4(*R*) hydrogen atoms. This finding is consistent with the poor observed enantioselectivity for substrate **1c** in the WT enzyme.

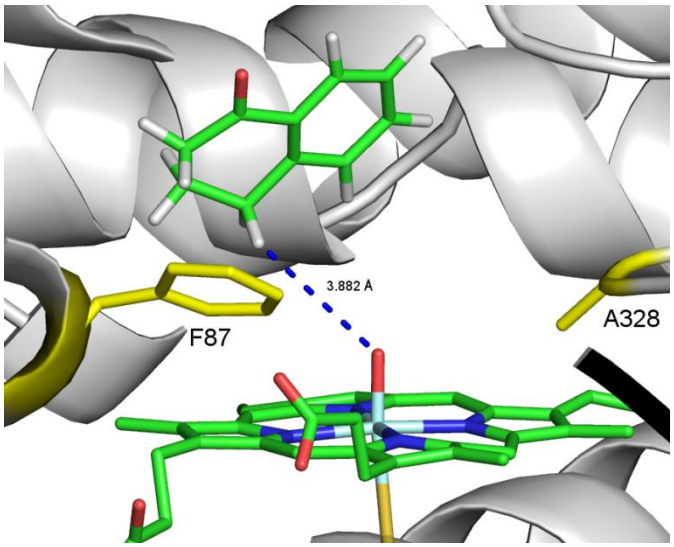


Fig. 1 Structure obtained from an unrestrained molecular dynamics simulation of 1-tetralone (**1a**) in WT P450-BM3 (after 34,940 ps). The O-H distance between the ferryl oxygen of Compound I and the pro-*S* hydrogen attached to C4 of 1-tetralone is highlighted by the blue dashed line. The F87 and A328 residues are also highlighted in yellow stick form.

Conclusions

In summary, we have developed an efficient biocatalytic one-step access to 4-hydroxy derivatives of 1-tetralone, many of which are important building blocks in the synthesis of biologically active natural products and therapeutic drugs. The approach described herein involves CH-activating oxidative hydroxylation of readily available 1-tetralone derivatives, catalysed by evolved mutants of P450-BM3 which ensure high degrees of regio- and stereoselectivity. This strategy is also successful in the oxidative hydroxylation of indane and tetralin, an approach which is currently not possible using chiral synthetic CH-activating transition metal catalysts.[8]

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Notes and references

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1 L. Liu, W. Li, K. Koike, S. Zhang and T. Nikaido, *Chem. Pharm. Bull.*, 2004, **52**, 566−569, and references therein.
2 Q. Liu, P. Zhao, X.-C. Li, M. R. Jacob, C.-R. Yang and Y.-J. Zhang, *Helv. Chim. Acta*, 2010, **93**, 265−271.
3 (*a*) H. Ito, T. Okuda, T. Fukuda, T. Hatano and T. Yoshida, *J. Agric. Food Chem.*, 2007, **55**, 672−679; (*b*) F.-S. Li, J. Shen and G.-S. Tan, *Chin. Tradit. Pat. Med.*, 2007, **29**, 1490.
4 C.-K. Woo and M. Bodil Van Niel, US patent application 0143914 A1 (2013).
5 Compound **2a** has been prepared by catalytic desymmetrization of meso-1,4-dihydroxytetralin using chiral Pd- or Ir-catalysts: (*a*) E. M. Ferreira and B. M. Stoltz, *J. Am. Chem. Soc.*, 2001, **123**, 7725−7726; (*b*) T. Suzuki, K. Ghozati, T. Katoh and H. Sasai, *Org. Lett.*, 2009, **11**, 4286−4288.
6 Reviews of P450 monooxygenases: (*a*) P. R. Ortiz de Montellano, *Cytochrome P450: Structure, Mechanism, and Biochemistry*, 3rd ed., Springer, Berlin, 2005; (*b*) E. M. Isin and F. P. Guengerich, *Biochim. Biophys. Acta Gen. Subj.*, 2007, **1770**, 314–329; (*c*) P. R. Ortiz de Montellano, *Chem. Rev.*, 2010, **110**, 932–948; (*d*) C. J. C. Whitehouse, S. G. Bell and L.-L. Wong, *Chem. Soc. Rev.*, 2012, **41**, 1218–1260; (*e*) E. O'Reilly, V. Kçhler, S. L. Flitsch and N. J. Turner, *Chem. Commun.*, 2011, **47**, 2490–2501; (*f*) R. Fasan, *ACS Catal.*, 2012, **2**, 647–666; (*g*) Y. Khatri, F. Hannemann, M. Girhard, R.

Kappl, A. Meme, M. Ringle, S. Janocha, E. Leize-Wagner, V. B. Urlacher and R. Bernhardt, *Biotechnol. Appl. Biochem*., 2013, **60**, 18–29; (*h*) F. Hollmann, D. Holtmann, M. W. Fraaije, D. J. Opperman and I. W. C. E. Arends, *Chem. Commun*., 2014, DOI: 10.1039/C3CC49747J.

7 Selected examples of protein engineering of P450 enzymes[10]: (*a*) Y. Yang, J. Liu, Z. Li, *Angew. Chem., Int. Ed*., 2014, **53**, 3120–3124; (*b*) F. Brühlmann, L. Fourage, L. Jeckelmann, C. Dubois, D. Wahler, *J. Biotechnol.*, 2014, **184**, 17–26; (*c*) B. M. A. van Vugt-Lussenburg, M. C. Damsten, D. M. Maasdijk, N. P. E. Vermeulen and J. N. M. Commandeur, *Biochem. Biophys. Res. Commun*., 2006, **346**, 810–818; (*d*) S. T. Jung, R. Lauchli and F. H. Arnold, *Curr. Opin. Biotechnol*., 2011, **22**, 809–817; (*e*) W. L. Tang, Z. Li and H. Zhao, *Chem. Commun*., 2010, **46**, 5461–5463; (*f*) P. R. Ortiz de Montellano, *Chem. Rev*., 2010, **110**, 932–948; (*g*) V. B. Urlacher and M. Girhard, *Trends Biotechnol*., 2012, **30**, 26–36; (*h*) K. L. Tee and U. Schwaneberg, *Comb. Chem. High Throughput Screening*, 2007, **10**, 197–217; (*i*) H. Venkataraman, S. B. A. de Beer, L. A. H. van Bergen, N. van Essen, D. P. Geerke, N. P. E. Vermeulen and J. N. M. Commandeur, *ChemBioChem*, 2012, **13**, 520–523; (*j*) J. C. Lewis, S. M. Mantovani, Y. Fu, C. D. Snow, R. S. Komor, C. H. Wong and F. H. Arnold, *ChemBioChem*, 2010, **11**, 2502–2505; (*k*) K. Zhang, S. E. Damaty and R. Fasan, *J. Am. Chem. Soc*., 2011, **133**, 3242–3245.

8 Synthetic transition metal catalysts for CH-activating oxidative hydroxylation: (*a*) T. Newhouse and P. S. Baran, *Angew. Chem., Int. Ed*., 2011, **50**, 3362–3374; (*b*) M. C. White, *Science*, 2012, **335**, 807–809; (*c*) S. R. Neufeldt and M. S. Sanford, *Acc. Chem. Res*., 2012, **45**, 936–946; (*d*) E. Roduner, W. Kaim, B. Sarkar, V. B. Urlacher, J. Pleiss, R. Gläser, W.-D. Einicke, G. A. Sprenger, U. Beifuß, E. Klemm, C. Liebner, H. Hieronymus, S.-F. Hsu, B. Plietker and S. Laschat, *ChemCatChem*, 2013, **5**, 82–112.

9 (*a*) L. O. Narhi and A. J. Fulco, *J. Biol. Chem*., 1986, **261**, 7160–7169; (*b*) A. W. Munro, D. J. Leys, K. J. McLean, K. R. Marshall, T. W. B. Ost, S. Daff, C. S. Miles, S. K. Chapman, D. A. Lysek, C. C. Moser, C. C. Page and P. L. Dutton, *Trends BioChem. Sci*., 2002, **27**, 250–257; (*c*) T. Jovanovic, R. Farid, R. A. Friesner and A. E. McDermott, *J. Am. Chem. Soc*., 2005, **127**, 13548–13552; (*d*) K. H. Clodfelter, D. J. Waxman and S. Vajda, *Biochemistry*, 2006, **45**, 9393–9407; (*e*) U. Schwaneberg, A. Sprauer, C. Schmidt-Dannert and R. D. Schmid, *J. Chromatogr. A*, 1999, **848**, 149–159.

10 (*a*) S. Kille, F. E. Zilly, J. P. Acevedo and M. T. Reetz, *Nat. Chem.*, 2011, **3**, 738–743; (*b*) R. Agudo, G.-D. Roiban and M. T. Reetz, *ChemBioChem*, 2012, **13**, 1465–1473; (*c*) G.-D. Roiban, R. Agudo and M. T. Reetz *Angew. Chem., Int. Ed.,* 2014, DOI: 10.1002/anie.201310892.

11 Recent reviews of directed evolution: (*a*) *Protein Engineering Handbook*, ed. S. Lutz and U. T. Bornscheuer, Wiley-VCH, Weinheim, vol. 1–2, 2009; (*b*) N. J. Turner, *Nat. Chem. Biol*., 2009, **5**, 567–573; (*c*) C. Jäckel, P. Kast and D. Hilvert, *Annu. Rev. Biophys*., 2008, **37**, 153–173; (*d*) S. Bershtein and D. S. Tawfik, *Curr. Opin. Chem. Biol*., 2008, **12**, 151–158; (*e*) P. A. Romero and F. H. Arnold, *Nat. Rev. Mol. Cell Biol*., 2009, **10**, 866–876; (*f*) L. G. Otten, F. Hollmann and I. W. C. E. Arends, *Trends Biotechnol*., 2010, **28**, 46–54; (*g*) A. V. Shivange, J. Marienhagen, H. Mundhada, A. Schenk and U. Schwaneberg, *Curr. Opin. Chem. Biol*., 2009, **13**, 19–25; (*h*) M. T. Reetz in *Asymmetric Organic Synthesis with Enzymes*, ed. V. Gotor, I. Alfonso and E. García-Urdiales, Wiley-VCH, Weinheim, 2008, pp. 21–63; (*i*) G. A. Strohmeier, H. Pichler, O. May and M. Gruber-Khadjawi, *Chem. Rev*., 2011, **111**, 4141–4164.

12 M. T. Reetz, *Angew. Chem., Int. Ed.*, 2011, **50**, 138–174.

13 S. M. Resnick, D. S. Torok, K. Lee, J. M. Brand and D. T. Gibson, *Appl. Environ. Microbiol*., 1994, **60**, 3323–3328.

14 (*a*) R. Noyori, *Angew. Chem., Int. Ed*., 2002, **41**, 2008–2022; (*b*) Teva Pharmaceutical Industries Ltd. US Pat., 2006/199974 A1, 2006; (*c*) M. Ito, Y. Shibata, A. Watanabe and T. Ikariya, *Synlett*, 2009, **10**, 1621–1626; (*d*) A. M. Teitelbaum, A. Meissner, R. A. Harding, C. A. Wong, C. C. Aldrich and R. P. Remmel, *Bioorg. Med. Chem.*, 2013, **21**, 5605–5617.

15 Biological activity for **6a**: (*a*) I. Bichlmaier, A. Siiskonen, M. Finel and J. Yli-Kauhaluoma, *J. Med. Chem*., 2006, **49**, 1818–1827; (*b*) Takeda Pharmaceutical Company Limited EP1559422 A1, 2005; EP 1559422 A1; (*c*) G. B. af Gennas, V. Talman, O. Aitio, E. Ekokoski, M. Finel, R. K. Tuominen and J. Yli-Kauhaluoma, *J. Med. Chem.*, 2009, **52**, 3969–3981; biological activity for **6b**: (*d*) D. G. Barrett, J. G. Catalano, D. N. Deaton, S. T. Long, R. B. McFadyen, A. B. Miller, L. R. Miller, K. J. Wells-Knecht and L. L. Wright, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 2209–2213; (*e*) Tibotec Pharmaceuticals Ltd WO2008/99019 A1, 2008.

16 (*a*) S. Shaik, D. Kumar, S. P. de Visser, A. Altun and W. Thiel, *Chem. Rev*., 2005, **105**, 2279–2328; (*b*) R. Lonsdale, J. N. Harvey and A. J. Mulholland, *J. Phys. Chem. Lett.*, 2010, **1**, 3232–3237; (*c*) R. Lonsdale, J. N. Harvey and A. J. Mulholland, *J. Phys. Chem. B*, 2010, **114**, 1156–1162.

