Appl Microbiol Biotechnol (2017) 101:987–1001
DOI 10.1007/s00253-016-8083-6

MINI-REVIEW

Recent advances in biotechnological applications of alcohol dehydrogenases

Yu-Guo Zheng[1] · Huan-Huan Yin[1] · Dao-Fu Yu[1] · Xiang Chen[1] · Xiao-Ling Tang[1] · Xiao-Jian Zhang[1] · Ya-Ping Xue[1] · Ya-Jun Wang[1] · Zhi-Qiang Liu[1]

Received: 11 November 2016 / Revised: 16 December 2016 / Accepted: 19 December 2016 / Published online: 10 January 2017

Abstract Alcohol dehydrogenases (ADHs), which belong to the oxidoreductase superfamily, catalyze the interconversion between alcohols and aldehydes or ketones with high stereoselectivity under mild conditions. ADHs are widely employed as biocatalysts for the dynamic kinetic resolution of racemic substrates and for the preparation of enantiomerically pure chemicals. This review provides an overview of biotechnological applications for ADHs in the production of chiral pharmaceuticals and fine chemicals.

Keywords Alcohol dehydrogenases · Biotechnological applications · Chiral pharmaceuticals · Fine chemicals

Introduction

Alcohol dehydrogenases (E.C. 1.1.1.*x*; *x* = 1 or 2, ADHs) are a group of oxidoreductases that catalyze the interconversion between alcohols and aldehydes or ketones by transferring C4-hydride from NAD(P)H to the carbonyl carbon of an aldehyde or ketone substrate (Musa and Phillips 2011). During the last decade, ADHs have been widely studied and applied in the asymmetric synthesis of chiral alcohols, based on their high stereoselectivity under mild conditions (Musa and Phillips 2011; Zhang et al. 2015). Although ADHs have various catalytic advantages, the widespread application of ADHs to the large-scale production of target products is still restricted to their commercial availability and the low aqueous solubility of the substrates. More attention has been paid to the exploration of robust enzymes with high catalytic efficiency and the development of novel non-aqueous media to improve the solubility of substrates (Carvalho 2016; Wang et al. 2016; Zhang et al. 2015).

In the past few years, a considerable number of publications on ADHs and the corresponding asymmetric biosynthesis have been documented and reviewed from various aspects. Hall and Bommarius (2011) exhaustively discussed the major advances of reductase-catalyzed and oxidase-catalyzed redox reactions and covered reduction, oxidation, and corresponding cofactor regeneration. Musa and Phillips (2011) provided an overview of ADH-catalyzed asymmetric synthesis of hydrophobic alcohols. Biocatalytic asymmetric reduction to chiral alcohols was also summarized (Patel 2013). Nealon et al. (2015) discussed the factors that influence the substrate stereospecificity and specificity of ADHs. Recently, Zhang et al. (2015) reviewed the rational and/or irrational design of ADHs and the methods for screening the desired ADH variants.

This review focuses on the latest advances in biological applications of ADHs in the preparation of chiral pharmaceutical intermediates and fine chemicals, followed by future aspects of ADH-related research to enhance the industrial applications.

Biological applications of ADHs in the production of chiral pharmaceutical intermediates

Antidepressants

(*S*)-Duloxetine, (*S*)-(+)-*N*-methyl-3-(1-naphthalenyloxy)-3-(2-thienyl)propanamine, is not only used as a new

✉ Yu-Guo Zheng
zhengyg@zjut.edu.cn

✉ Zhi-Qiang Liu
microliu@zjut.edu.cn

[1] Key Laboratory of Bioorganic Synthesis of Zhejiang Province, College of Biotechnology and Bioengineering, Zhejiang University of Technology, Hangzhou 310014, People's Republic of China

antidepressant and a dual inhibitor of serotonin and norepinephrine reuptake but is also regarded as a potential drug for the treatment of stress urinary incontinence. The chemical synthesis of (*S*)-duloxetine was first described by Eli Lilly using a stereoselective reduction of prochiral ketones **1a**–**1f** to the (*S*)-chiral alcohols **2a**–**2f** as the key step (Fig. 1). The chiral metal ligand catalysts used in this step are costly, and the process resulted in trace metal contamination in the products. ADH-catalyzed processes for reduction of the ketones **1a**–**1f** were developed to synthesize (*S*)-duloxetine to avoid the shortcomings of the chemical methods (Chen et al. 2016). Whole *Candida tropicalis* PBR 2 cells were first used for the asymmetric reduction of compound **1b** to produce compound (*S*)-**2b** with 80% product yield and >99% enantiometric excess (*ee*). Furthermore, whole *Candida viswanathii* cells were used for the gram-scale reduction of compound **1b** to compound (*S*)-**2b** at >81% conversion and >99% *ee* (Soni et al. 2008). Recently, the *Lactobacillus kefir* ADH was used as a template, and a series of ADH variants were obtained through protein engineering by Codexis (Redwood City, CA, USA) that reduced compound **1a** to compound (*S*)-**2a** and compound **1b** to compound (*S*)-**2b**. Using the isopropanol-based cofactor regeneration, the concentration of substrate was up to 150 g/L and the product yield and *ee* were 90 and >99%, respectively, which opened the door for the bioproduction of (*S*)-duloxetine (Savile et al. 2014).

Befloxatone, (*5R*)-5-(methyloxymethyl)-3-[4-(*3R*)-4,4,4-trifluoro-3-hydroxybutoxy]phenyl-2-oxazalidinone, a novel oxazolidinone derivative, has been introduced into clinical practice as the first potent antidepressant drug which selectively and competitively inhibits monoamine oxidase (MAO)-A. (*R*)-Ethyl 3-hydroxy-4,4,4-trifluorobutanoate ((*R*)-EHTB) was regarded as the common intermediate in befloxatone synthesis. The chemical synthesis of (*R*)-EHTB from ethyl 3-oxo-4,4,4-trifluorobutanoate (EOTB) requires two steps involving $NaBH_4$-dependent reduction and a subsequent kinetic resolution, which was constructed by Novozyme (Bagsvaerd, Denmark) (Tixidre et al. 1998). Due to the low yield in the chemical process, the biocatalyst-based asymmetric reduction of ketone EOTB to (*R*)-EHTB was developed using permeabilized *Bacillus pumilus* Phe-C3 cells containing an NADPH-dependent ADH and a G-6-PDH, with 67% product yield and 95% *ee* at a substrate concentration of 60 mM (Zhang et al. 2006). Whole *Saccharomyces uvarum* SW-58 cells were further used in an aqueous-organic solvent biphasic system for the asymmetric reduction of ketone EOTB to (*R*)-EHTB with 85.0% conversion and 85.2% *ee* (He et al. 2007). Whole *Kluyveromyces marxianus* cells also have the ability to catalyze EOTB to (*R*)-EHTB, with a relatively low yield (81%) and *ee* (24%) (Oliveira et al. 2013). Recently, Kara et al. (2014) established a highly productive biocatalytic reduction route for EOTB to (*R*)-EHTB using an isolated ADH in non-aqueous media, and a high conversion of 99.9% was obtained, with >99.9% *ee*. These studies make it possible for (*R*)-EHTB to be produced using the biocatalytically asymmetric reduction method.

Antianxiety drugs

Buspirone (Buspar®), a potent serotonin $5HT_{1A}$ receptor partial agonist, is used for the treatment of generalized anxiolytic disorder. It could bind to D_3 and D_4 receptors with high affinity and is applied to treat cocaine self-administration. Notably, 6-hydroxybuspirone is a major metabolite of buspirone and contributes to the effectiveness of buspirone, due to its high plasma concentration. There is no apparent distinction between (*R*)-6-hydroxybuspirone and (*S*)-6-hydroxybuspirone, showing that 6-hydroxybuspirone is effective and could be developed as a potential anxiolytic agent. The enantioselective microbial reduction of 6-oxo-8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4,5]decane-7,9-dione (6-ketobuspirone) to either (*R*)-6-hydroxybuspirone or (*S*)-6-hydroxybuspirone has been developed. Patel et al. (2005) screened approximately 150 strains for the enantioselective reduction of 6-ketobuspirone, where whole *Rhizopus*

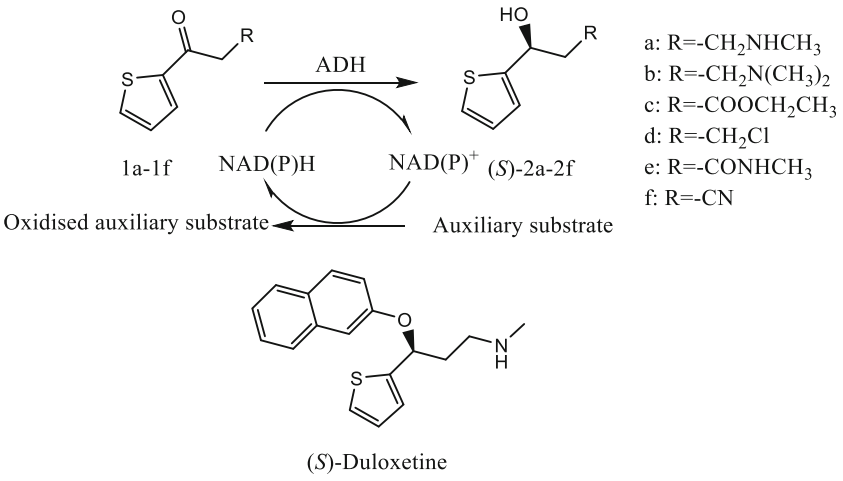

Fig. 1 Bioreduction process for synthesis of (*S*)-duloxetine intermediates (Tang et al. 2011)

stolonifer SC 13898 cells showed the capability of producing (*S*)-6-hydroxybuspirone, with >53% yield and >96.6% *ee*. Meanwhile, the strain *Candida maltose* SC 16112 exhibited the ability to produce (*R*)-6-hydroxybuspirone, with >57% yield and >97% *ee*. A NADPH-dependent ADH from *Hansenula polymorpha* SC 13845 was expressed together with a G-6-PDH from *Saccharomyces cerevisiae* and catalyzed the reduction of 6-ketobuspirone to (*R*)-6-hydroxybuspirone with a 99% yield and 99.9% *ee* at 50 g/L substrate (Goldberg et al. 2006). In addition, an ADH isolated from *Pseudomonas putida* SC 16269 was combined with formate dehydrogenase from *Pichia pastoris* and reduced 50 g/L 6-ketobuspirone to (*S*)-6-hydroxybuspirone with a >98% yield and >99.9% *ee* (Goldberg et al. 2006).

Antiasthmatics

Montelukast, (*2S*)-3-[[(*1S*)-3-(2-acetylphenyl)-1-[3-[(*1E*)-2-(7-chloro-2-quinolinyl)ethenyl]phenyl]propyl]thio]-2-methylpropanoic acid, was identified by Merck as a cysteinyl leukotriene 1 receptor antagonist and developed as a therapeutic agent for treating asthma. Merck described the chemical synthetic route for the production of montelukast using a stereospecific reduction of (*E*)-2-(3-(3-(2-(7-chloro-2-quinolinyl)ethenyl)phenyl)-3-oxopropyl)benzoic acid methyl ester (keto ester M) to (*S,E*)-methyl 2-(3-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-hydroxopropyl)benzoic acid methyl ester ((*S*)-hydroxy ester) as the key step. Because there were several disadvantages of the chemical methods, enzyme-catalyzed processes for this reduction were explored. The first successful reaction used whole *Microbacterium* sp. MB 5614 cells containing a ketoreductase for the synthesis of (*S*)-hydroxy ester with >95% *ee* (Roberge et al. 1996). Codexis engineered *L. kefir* ADH through directed enzyme evolution for the asymmetric reduction of keto ester M to the corresponding (*S*)-hydroxy ester. After a series of mutations, one mutant was finally obtained exhibiting excellent productivity of (*S*)-hydroxy ester, which was improved by 2000-fold and had high enantioselectivity and stability. The final yield and *ee* reached 99.3 and 99.9%, respectively, at a substrate concentration of 100 g/L (Liang et al. 2012).

(*R*)-(−)-3-Quinuclidinol, which contains a double-ring structure with nitrogen, is a valuable chiral building block for pharmaceuticals such as talsaclidine and revatropate, which are used as bronchodilator agents. Several routes have been reported for the preparation of (*R*)-(−)-3-quinuclidinol, including the resolution of racemic (*R*)-(−)-3-quinuclidinol and asymmetric reduction of 3-quinuclidinone (Tsutsumi et al. 2009). More attention was paid to developing the direct reduction of 3-quinuclidinone to (*R*)-(−)-3-quinuclidinol by ADH, with a maximum theoretical yield of 100%, to break through the bottleneck of 50% theoretical yield in the racemic resolution approach. A carbonyl reductase from *Rhodotorula rubra* JCM3782 was coexpressed with glucose dehydrogenase (GDH) for cofactor regeneration and catalyzed the reduction of 3-quinuclidinone (618 mM) to (*R*)-(−)-3-quinuclidinol with 98% conversion and >99.9% *ee* (Uzura et al. 2009). A new strain, *Nocardia* sp. WY1202, was isolated for the stereospecific reduction of 3-quinuclidinone to produce (*R*)-(−)-3-quinuclidinol with a 93% yield and >99% *ee* (Wang et al. 2013a). In addition, two novel reductases (QNR and bacC) that were capable of reducing 3-quinuclidinone to (*R*)-(−)-3-quinuclidinol were cloned from *Microbacterium luteolum* JCM 9174 and coexpressed with an ADH from *Leifsonia* sp. (LSADH) for cofactor regeneration. As a result, 939 mM 3-quinuclidinone was converted to (*R*)-(−)-3-quinuclidinol, with a conversion of 100 and 99.9% *ee*, by immobilized *Escherichia coli* cells containing recombinant QNR (Isotani et al. 2012). Recently, Zhang et al. (2013b) described a new ketoreductase (ArQR) from *Agrobacterium radiobacter* ECU2556 that was capable of catalyzing the conversion of >200 g/L substrate 3-quinuclidinone to (*R*)-(−)-3-quinuclidinol with 99% *ee* and a high space-time yield of up to 916 g/L/day, which is competitive for biomanufacturing (*R*)-(−)-3-quinuclidinol on an industrial scale.

Anticholesterol drugs

Statins, also known as hydroxymethylglutaryl CoA (HMG CoA) reductase inhibitors, can reduce low-density lipoprotein cholesterol (LDL-C) by inhibiting liver cholesterol synthesis and are regarded as one of the top-selling drugs for hypercholesterolemia treatment. (*R*)-4-Cyano-3-hydroxybutyrate ((*R*)-HN) is a key intermediate for the production of atorvastatin and can be converted to the appropriate side chain upon Claisen condensation and reduction. An enzymatic process for the preparation of (*R*)-HN from ethyl 4-chloro-3-oxobutanoate (COBE) was developed by Codexis using a two-enzyme system with a ketoreductase and halohydrin dehalogenase (HHDL) (Davis et al. 2010). For this process, the stereospecific reduction of COBE to (*S*)-ethyl 4-chloro-3-hydroxybutanote ((*S*)-CHBE) was achieved by reductases from *Geotrichum candidum*, *Pichia stipitis*, *Candida parapsilosis* ATCC 7330, and other previously reported strains (Kaliaperumal et al. 2011; Liu et al. 2015; Patel et al. 1992; Ye et al. 2011; You et al. 2014). A substrate-coupled biocatalytic process driven by a NADPH-dependent sorbose reductase from *Candida albicans* was described to convert 2.5 M COBE to (*S*)-CHBE, with a 93.6% yield and 99.6% *ee* (Cai et al. 2012). In addition, highly efficient synthesis of (*S*)-CHBE was developed with whole *E. coli* BL21 cells containing a reductase gene cloned from *Streptomyces coelicolor*, and the reaction used 2-propanol for cofactor regeneration in a water/toluene biphasic system. As much as 3.6 M COBE was asymmetrically reduced within 22 h, resulting in a 93% yield and >99% *ee* (Wang et al. 2011a). Recently, novel reductases

identified by genome mining were applied to produce (*S*)-CHBE with a high concentration of COBE (3 M), a high yield (>99.0%), and a high *ee* value (>99.9%), indicating their great potential in the industrial production of (*S*)-CHBE (You et al. 2014; Liu et al. 2015).

As shown in Fig. 2, compounds **6a**–**6d** are the key chiral precursors for HMG-CoA reductase inhibitors that are synthesized by stereoselective reduction of compounds **3a**–**3d**, which contain two carbonyl groups. Using a suspension of *Acinetobacter calcoaceticus* SC 13876 cells as the biocatalyst, both compounds **3a** and **3b** were stereospecifically reduced to the corresponding (*3R*)-**5a**–**5b** and (*5S*)-**4a**–**4b** compounds, followed by further reduction to the corresponding dihydroxy esters (*3R,5S*)-**6a** and (*3R,5S*)-**6b**. The desired (*3R,5S*)-**6a** compound was obtained with a 99% *ee* and 97% diastereomeric excess (*de*), whereas the (*3R,5S*)-**6b** compound was produced with 99% *ee* and 63% *de* (Guo et al. 2006). Subsequently, Goldberg et al. (2008) cloned and identified a desired ketoreductase III from *A. calcoaceticus* SC 13876 that catalyzed the stereoselective reduction of ketoesters **4b** to (*3R,5S*)-**6b** with 99.3% yield, 100% *ee*, and 99.8% *de*. In addition, a purified diketoreductase (DKR) from *Acinetobacter baylyi* ATCC 33305 was described to stereoselectively reduce compound **4a** to compound **6a**, with both *de* and *ee* values greater than 99.5% (Wu et al. 2009). Recently, Wang et al. (2015) and Luo et al. (2015) cloned and expressed CaAKR from *C. albicans* XP1463 and KlAKR from *Kluyveromyces lactis* XP1461, respectively, to catalyze the stereoselective reduction of ketoester **4c** to compound (*3R,5S*)-**6c** with both *de* and *ee* values greater than 90 and 99% for CaAKR and KlAKR, respectively. Furthermore, 11.25-fold higher catalytic efficiency (*k*cat/*K*m) of KIAKR towards compound **4c** was achieved through two rounds of site saturation mutagenesis (Luo et al. 2016). Different cofactor regeneration systems and an aqueous-organic biphasic reaction system were evaluated for this biocatalytic process using purified recombinant DKR. An aqueous-hexane biphasic system was further scaled up to 0.5 L at a substrate concentration of 105 g/L, and the product was obtained with 83.5% yield, >95.5% *de*, and >99.5% *ee* (Wu et al. 2011). In addition, Codexis developed a series of mutants from a *S. cerevisiae* ketoreductase using a directed enzyme evolution method, and the final mutant was isolated to catalyze the reduction of both compounds **4c** and **4d** to compounds (*3R,5R*)-**6c**–**6d** or (*3R,5S*)-**6c**–**6d** with 99.9% *ee* and 99.99% *de* at a substrate concentration of 150 g/L (Sun et al. 2013). These biocatalytic approaches avoided most of the disadvantages that occurred in the chemical methods, which paved the foundation for the practical synthesis of statin drugs.

Ezetimibe (Zetia® and Ezetrol®) is a novel cholesterol absorption inhibitor that decreases the LDL-C levels by binding and thereby inhibiting the function of Niemann-Pick C1-Like 1 (NPC1L1), the main cholesterol absorption transporter in the intestine. The key step of ezetimibe synthesis involves the asymmetric reduction of 1-(4-fluorophenyl)-5-(2-oxo-4-phenyl-oxazolidin-3-yl)-pentane-1,5-dione (FOP dione) to 3-[5-(4-fluorophenyl)-5(*S*)-hydroxypentanoyl]-4(*S*)-4-phenyl-1,3-oxazolidin-2-one ((*S*)-FOP alcohol). The microbial enantioselective reduction of FOP dione to (*S*)-FOP alcohol with >99% *ee* was initially performed by whole *Schizosaccharomyces octosporus* ATCC 2479 and *Burkholderia cenocepacia* MTCC 5427 cells with 54 and 34% yield, respectively (Homann and Previte 1997; Singh et al. 2009). Subsequently, Codexis developed a series of engineered ketoreductases from *L. kefir*, *Lactobacillus brevis*, and *Lactobacillus minor* for the reduction of FOP dione to (*S*)-FOP alcohol, and one mutant with improved properties was screened and employed to produce (*S*)-FOP alcohol at a preparative scale in the presence of 100 g/L substrate. The yield was approximately 99%, and the stereomeric purity was >99% (Mundorff and De Vries 2013).

Antihypertensive drugs

Ethyl (*R*)-(−)-2-hydroxy-4-phenylbutyrate ((*R*)-**8**) (Fig. 3) is a versatile intermediate for the production of angiotensin-converting enzyme (ACE) inhibitors, including benazepril, lisinopril, and enalapril, which are widely used to treat

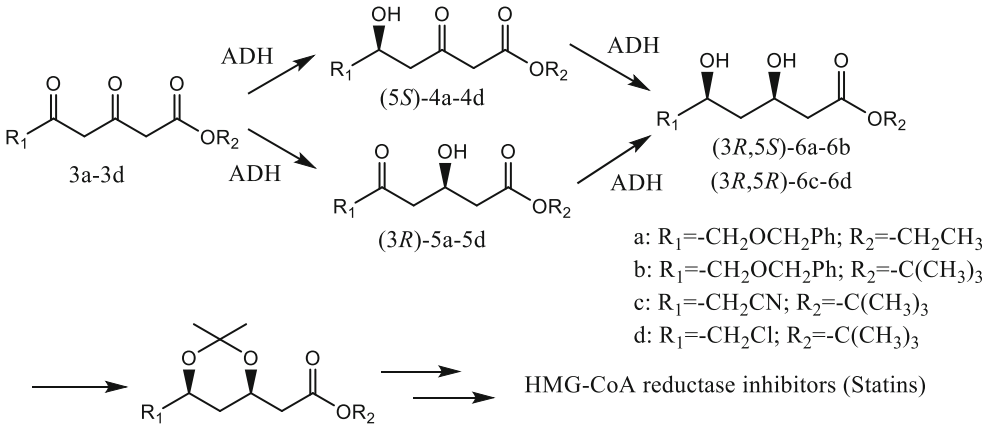

Fig. 2 Bioreduction process for synthesis of HMG-CoA reductase inhibitor intermediates (Goldberg et al. 2008; Guo et al. 2006)

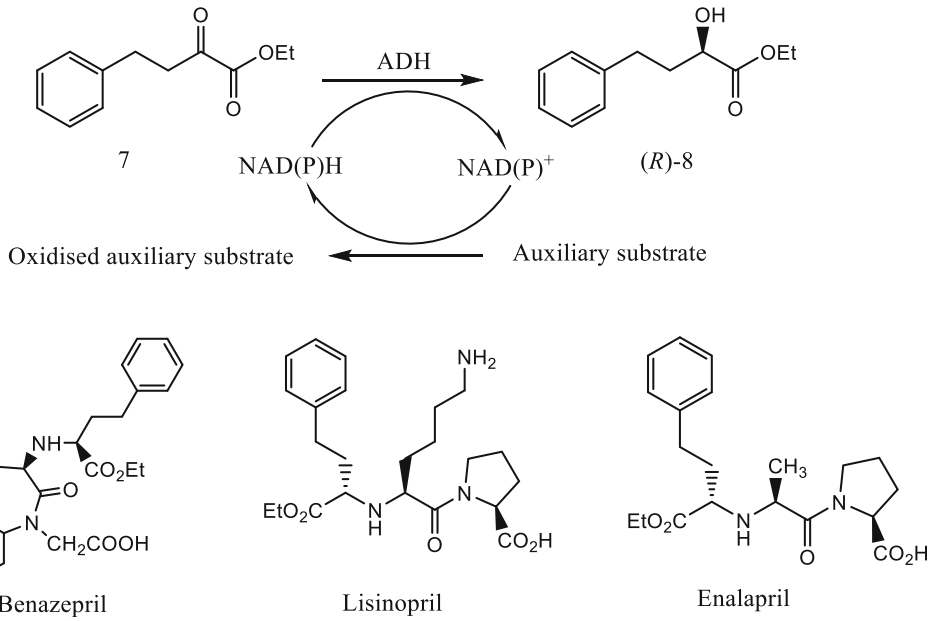

Fig. 3 Bioreduction process for synthesis of benazepril, lisinopril, and enalapril intermediate (Qian et al. 2014)

hypertension. Different approaches for (*R*)-**8** synthesis were explored, including the kinetic resolution of racemic **8**, asymmetric reduction of ethyl 2-oxo-4-phenylbutyrate (**7**), and multi-step synthesis (D'Arrigo et al. 2010). Biocatalytic asymmetric reduction was used as an attractive approach for the synthesis of compound (*R*)-**8**. A large number of microorganisms, including *Candida boidinii* CIOC21, *Candida krusei* SW2026, *Rhodotorula mucilaginosa* CCZU-G5, and *Candida magnoliae* CGMCC 2.1919, were shown to be the highly enantioselective biocatalysts in this reduction process (>99% *ee*) (Chen et al. 2008; Wang et al. 2013b; Xia et al. 2013; Zhang et al. 2009). Li et al. (2010) identified and characterized a highly enantioselective carbonyl reductase from *C. krusei* SW 2026, which was responsible for the reduction of compound **7**. Furthermore, whole *E. coli* cells containing recombinant *β*-ketoacyl-ACP reductase (FabG) from *Bacillus* sp. ECU0013 were reported to reduce up to 620 g/L of compound **7** to compound (*R*)-**8** with >99% *ee* (Ni et al. 2011), suggesting that FabG is a promising biocatalyst for the production of compound (*R*)-**8**.

Substrate feeding approaches and an aqueous/organic biphasic system were successfully constructed to improve the substrate tolerance and enhance the production of compound (*R*)-**8**. Ni et al. (2013) and Su et al. (2012) developed a 1 L aqueous/octanol biphasic reaction system for the synthesis of compound (*R*)-**8** with a recombinant *E. coli* strain that coexpressed a novel carbonyl reductase and GDH, resulting in a space-time yield of 660 g/L/day and 99.5% *ee* at a substrate concentration of 330 g/L. Recently, a D-lactate dehydrogenase mutant from *Lactobacillus bulgaricus* ATCC 11842 was reported to efficiently catalyze the reduction of compound **7** to compound (*R*)-**8** with high productivity (47.9 mM/h) and high enantioselectivity (>99% *ee*) when it was coexpressed with formate dehydrogenase in *E. coli* (Sheng et al. 2014). In addition, Qian et al. (2014) constructed a recombinant *P. pastoris* to express carbonyl reductase CgKR2 from *Candida glabrata* for the stereoselective reduction of compound **7** to compound (*R*)-**8**, achieving a complete conversion of 1.0 M compound **7** at 0.5 L scale with 77.9% yield and 97.3% *ee*. These studies cumulatively represent the industrial process for producing compound (*R*)-**8** with a high conversion, *ee*, and yield.

Antithrombotic drugs

(*S*)-Clopidogrel (Plavix®), a platelet aggregation inhibitor, is widely used to treat heart attack or stroke caused by the formation of blood clots. To date, several synthetic routes, such as hydrolysis of a racemic nitrile compound, fractional crystallization from the racemic mixture, and asymmetric reduction of methyl *o*-chlorobenzoylformate (CBFM), have been developed for the preparation of methyl (*R*)-*o*-chloromandelate ((*R*)-CMM), the key chiral synthon for (*S*)-clopidogrel (Ma et al. 2012). The asymmetric reduction of CBFM to prepare (*R*)-CMM was considered as a more straightforward and attractive method, which could realize 100% theoretical yield. Whole *S. cerevisiae* cells were reported to reduce CBFM to (*R*)-CMM with 96.1% *ee* (Jeong et al. 2010). A purified ADH from *Thermus thermophilus* was identified and employed in the effective reduction of CBFM, resulting in a 95% yield and 92% *ee* using a GDH for cofactor regeneration (Pennacchio et al. 2011). Using genome mining, a novel carbonyl reductase, CgKR1, from *C. glabrata* was identified and used for the stereospecific reduction of CBFM to (*R*)-CMM. When combined with GDH for cofactor generation, up to 300 g/L CBFM was almost completely converted

to (*R*)-CMM, with 98.7% *ee* (Ma et al. 2012). The CBFM concentration was further improved to 500 g/L using whole *E. coli* cells that coexpressed the aldo-ketoreductase from a *Bacillus* sp. and GDH, and excellent productivity as high as 812 g/L/day with 88% yield and 99% *ee* was obtained at 20 °C in a 50-g scale without the external addition of expensive cofactor (Ni et al. 2012). In addition, Xu et al. (2014) described that glycerol improved the thermal and operational stability of an NADPH-dependent aldo-ketoreductase (YtbE), by which the substrate conversion was increased from 62.9 to 98.7% and 70.5 to 96.6% at 0.1 and 1.0 M CBFM, respectively.

Thrombin, a trypsin-like protease enzyme, plays a critical role in the production of a thrombus and thrombin inhibitors and was developed for venous thromboembolism therapy. (*R*)-3,3-Dimethyl-2-hydroxybutyric acid ((*R*)-DHBA) is a key chiral intermediate of the thrombin inhibitor. An enzymatic process was constructed for the asymmetric reduction of ethyl 3,3-dimethyl-2-oxobutanoate to (*R*)-DHBA using commercially available ketoreductase KRED1001, together with a GDH, which was used for cofactor regeneration. (*R*)-DHBA was isolated and saponified to the corresponding enantiomerically pure hydroxyl acid with 82% yield and 99.5% *ee* (Nelson et al. 2004).

Antiepileptic drugs

Talampanel, a non-competitive antagonist of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, is a novel therapeutic drug for treating epilepsy. (*S*)-α-Methyl-1,3-benzodioxole-5-ethanol ((*S*)-MBE) is an important chiral building block for the synthesis of talampanel. The stereoselective bioreduction of 3,4-methylenedioxyphenylacetone (MDA) to (*S*)-MBE was regarded as the promising route for the scale-up production of (*S*)-MBE and was initially performed using whole *Zygosaccharomyces rouxii* and *Debaryomyces hansenii* cells, both of which had a >90% yield and >99% *ee* (Erdélyi et al. 2006). Furthermore, a series of ketoreductases were used to prepare (*S*)-MBE, where purified horse liver alcohol dehydrogenase (HLADH) from horse liver, CPADH from *C. parapsilosis*, and RS-1 ADH from *Rhodococcus* species-1 exhibited high activities and high enantioselectivities. HLADH showed a 99% conversion and 95% *ee*, while CPADH and RS-1 ADH showed 99% conversion and 99% *ee* (Broussy et al. 2008). Itoh et al. (2012) described an efficient method for producing (*S*)-MBE by the asymmetric reduction of MDA in an isopropanol-water medium with a recombinant ADH from *Leifsonia* sp. S749 (LSADH), resulting in a 73% yield and >99% *ee*. Recently, Simon et al. (2014) investigated various ADHs for the stereoselective bioreduction of MDA, where crude ADH-A from *Rhodococcus ruber* and purified ADH-T from *Theromanaerobacter* were proved to be the best enzymes for the preparation of (*S*)-MBE with high *ee* (>99%) and good conversion (>96%). Moreover, these biotransformations were performed on a preparative scale (75 mM), and the synthesis of (*S*)-MBE was achieved in optically pure form (>99%) and obtained with an approximately 95% yield.

β-Lactam antibiotics

Penems and carbapenems, next-generation β-lactam antibiotics, are considered as the "first-line drugs" for the treatment of severe nosocomial infections due to their broad antibacterial spectra, antibacterial activities, and stabilities to most β-lactamases. 4-Aceoxyazetidinone, [(*2R,3R*)-3-(*R*)-1-(tert-butyldimethylsilyloxy)ethyl)-4-oxoazetidin-2-ylacetate], is a critical chiral intermediate for the production of penems and carbapenems, where (*R*)-1,3-butanediol ((*R*)-**10**) is a key starting material for producing 4-aceoxyazetidinone derivatives (Fig. 4a). The biocatalytic process for the preparation of compound (*R*)-**10** was achieved by two approaches, stereospecific oxidation of the undesired compound (*S*)-**10** to the racemate and asymmetric reduction of 4-hydroxy-2-butanone (**9**). Whole *C. parapsilosis* IFO 1396 and *K. lactis* IFO 1267 cells were identified for the resolution of racemate **9** to produce (*R*)-**10** and (*S*)-**10**, with an optical purity of 97% for *C. parapsilosis* IFO 1396 and 99% for *K. lactis* IFO 1267 (Matsuyama et al. 2001). A novel (*S*)-specific ADH (CpSADH) from *C. parapsilosis* was characterized. The activity of recombinant CpSADH expressed in *E. coli* was more than 2-fold higher than the wild-type whole *C. parapsilosis* cells, resulting in a higher production yield of compound (*R*)-**10**. Under the optimized conditions, the yield of compound (*R*)-**10** reached 72.6 g/L, with a molar recovery yield of 48.4% from a 15% racemate reaction mixture and an optical purity of 95% *ee* (Yamamoto et al. 2002). Furthermore, the enzyme-catalyzed asymmetric reduction of compound **9** to compound (*R*)-**10** was achieved by a newly isolated strain, *C. krusei* ZJB-09162, which exhibited a 83.9% conversion with a substrate concentration of 45.0 g/L and produced 38.7 g/L compound (*R*)-**10** with 99.0% *ee* (Zheng et al. 2012).

(*2S,3R*)-2-Benzamidomethyl-3-hydroxybutyrate derivatives **11a**–**11c** (Fig. 4b) are also key intermediates in the synthesis of 4-aceoxyazetidinone. The production of enantiomers (*2S,3R*)-**12a**–**12c** has previously been performed using ruthenium-catalyzed asymmetric reduction of the racemic compounds **11a**–**11c**, which required expensive ruthenium catalysts and harsh reaction conditions (Noyori et al. 1989). Biocatalysis-based asymmetric reduction of **11a**–**11c** has been developed to overcome these disadvantages. Cultured plant cells from *Parthenocissus tricuspidata* and *Gossypium hirsutum* were previously reported to convert compounds **11a** and **11b** to the corresponding (*2R,3S*) and (*2S,3S*) configurations with high stereoselectivities. The reduction by *P. tricuspidata* cells produced compound (*2R,3S*)-**12a** with a 97% yield, 100% *de*, and >99% *ee*. On the other hand, the

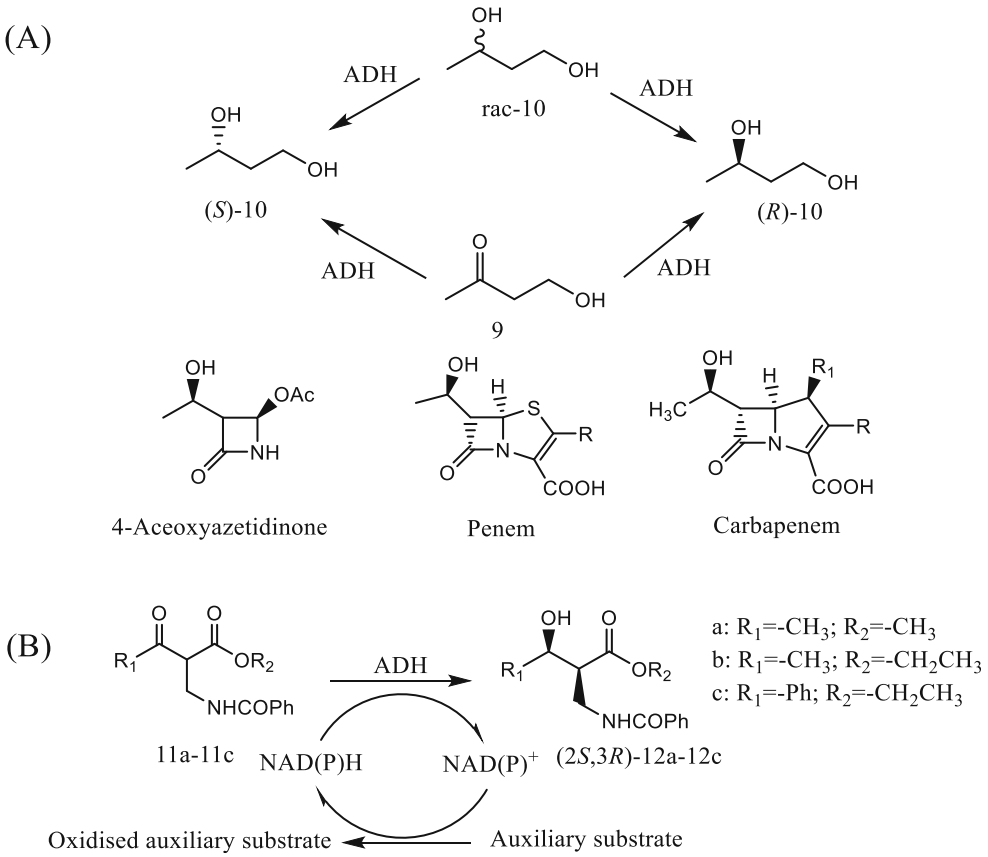

Fig. 4 Bioreduction process for synthesis of 4-aceoxyazetidinone intermediate (Gandolfi et al. 2009; Matsuyama et al. 2001; Rimoldi et al. 2011)

reduction with *G. hirsutum* cells exhibited a (*2S,3S*)-selectivity to produce compound (*2S,3S*)-**12a** with a 98% yield, 84% *de*, and >99% *ee* (Shimoda et al. 2009). Furthermore, the yeast strains *K. marxianus* var. lactis CL69 and *Pichia glucozyma* CBS 5766 were investigated in the stereoselective reduction of compound **11b**. Under the optimized conditions, whole *K. marxianus* var. lactis CL69 cells produced compound (*2R,3S*)-**12b** with >99% *ee* and 98% *de*, whereas whole *P. glucozyma* CBS 5766 cells produced compound (*2S,3S*)-**12b** with >99% *ee* and 86% *de* (Gandolfi et al. 2009). In addition, the reduction of compound **11c** to compound **12c** was achieved using whole *P. glucozyma* CBS 5766 and *K. marxianus* CBS 1553 cells, with high yields and stereoselectivity. The former generated compound (*2R,3S*)-**12c** with >99 *ee* and 70% *de*, whereas the latter furnished the enriched diastereoisomer (*2R,3R*)-**12c** with >99 *ee* and 88% *de* (Rimoldi et al. 2011). Notably, Codexis described a synthetic route for compound **11a** using ADHs from *L. brevis* and *Devosia riboflavin* as catalysts, providing compound (*2S,3R*)-**12a** with >99% *ee* and >89% *de* (Yasohara et al. 2009). Chen et al. (2015) also discovered bgADH2 from the SDR-producing strain *Burkholderia gladioli* and showed that it could be used for the asymmetric synthesis of compound (2*S*,3*R*)-**12a** with 99% *ee* and 98.5% *de*.

(*R*)-3-Hydroxythiolane is a key intermediate in sulopenem, and its original production process was developed by Pfizer (Volkmann et al. 1992). Codexis successfully applied enzyme evolution technologies a KRED, resulting in unprecedented high enantioselectivity (>99% *ee*) for reduction of tetrahydrothiophene-3-one to (*R*)-3-hydroxythiolane. This process has been successfully scaled up to 100 kg level and applied to the industrial production (Liang et al. 2009).

Non-steroidal anti-inflammatory drugs

Profens are a subclass of non-steroidal anti-inflammatory drugs, which are non-selective inhibitors of the cyclooxygenase involved in prostaglandin synthesis via the arachidonic acid pathway. (2*S*)-2-Arylpropanols (**14a–14f**) can be oxidized to (2*S*)-2-arylpropionic acids, which are used as key intermediates in the synthesis of Profens. Compounds **14a** and **14b** (Fig. 5) were obtained with high yields and enantiomeric ratios using HLADH as the catalyst through dynamic kinetic resolution in organic solvents or in phosphate-buffered aqueous solution (Giacomini et al. 2007). Purified HLADH was further used to prepare compounds **14a–14f** by the bioreduction of 2-arylpropionic aldehydes (**13a–13f**) under mild conditions (Galletti et al. 2010). High yields and high enantioselectivity towards the (*S*)-enantiomer were achieved in a dynamic kinetic resolution process by coupling the yeast ADH-catalyzed or HLADH-catalyzed reduction with a chemical base-catalyzed racemization of the unreacted aldehydes,

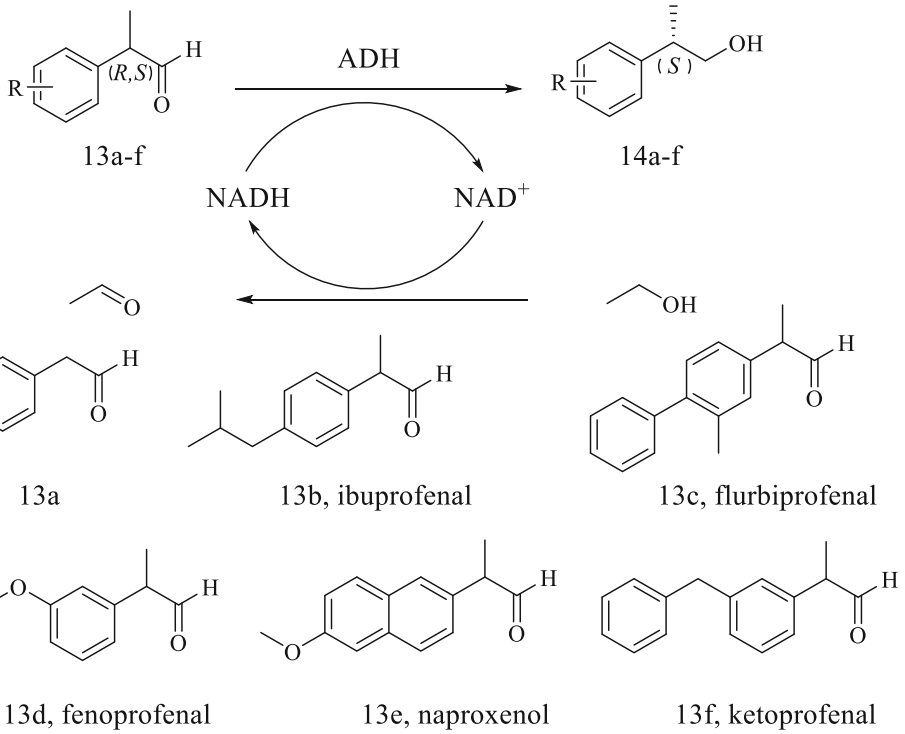

Fig. 5 Bioreduction process for synthesis of profen intermediates (Galletti et al. 2010; Friest et al. 2010; Quaglia et al. 2013)

which is a promising alternative process and can overcome the 50% yield limitation in classical kinetic resolution (Galletti et al. 2010). Recently, a histidine-tagged HLADH was successfully purified and immobilized for the improvement of enantioselectivity and reusability (Quaglia et al. 2013). Purified SsADH-10 from hyperthermophile *Sulfolobus solfataricus* was also employed in the dynamic kinetic resolution to produce compounds **14a–14f** with high *ee* and a preference for the (*S*)-enantiomer (Friest et al. 2010).

Anticancer drugs

Taxol (Paclitaxel®) is an anticancer drug with great potential that was approved by FDA to treat ovarian and breast cancers by blocking microtubule disassembly. (*2R,3S*)-*N*-Benzoyl-3-phenyl isoserine ethyl ester ((*2R,3S*)-**16**), a chiral intermediate of the C-13 side chain of taxol, was prepared by the stereoselective reduction of 2-keto-3-(*N*-benzoylamino)-3-phenyl propionic acid ethyl ester (**15**) (Fig. 6). The stereoselective reductions were performed by whole *H. polymorpha* 13865 and *Hansenula fabianii* 13894 cells, and product yield >80% and *ee* >98% were ultimately obtained (Patel et al. 1995). Yeast-catalyzed reduction of compound **15** to generate compound (*2R,3S*)-**16** was also reported (Kearns and Kayser 1994). A new ADH cloned from *Clostridium acetobutylicum* was used for the asymmetric reduction of 3-phenyl-2-chloro-3-oxopropionic acid ethyl ester (**17**), providing a building block (2*S*,3*R*)-2-chloro-3-hydroxy-3-phenylpropionic acid ethyl ester ((2*S*,3*R*)-**18**) with 95% yield, 95% *de*, and 99% *ee* (Applegate et al. 2011).

Crizotinib (PF-2341066), a dual inhibitor of the mesenchymal-epithelial transition (MET) and anaplastic lymphoma kinase (ALK), was approved by the FDA for the treatment of advanced non-small cell lung cancer (NSCLC). (*S*)-1-(2,6-Dichloro-3-fluorophenyl)-ethanol ((*S*)-DFPE) is a key intermediate required for the preparation of crizotinib. (*S*)-DFPE can be synthesized by enzymatic resolution, chemical esterification, and stereoselective bioreduction of 2,6-dichloro-5-fluorophenacetone (DFPA). Codexis developed a series of engineered ketoreductases from *L. kefir* ADH (ADH-LK) and *L. brevis* ADH (ADH-LB) to convert DFPA to (*S*)-DFPE, with >99% *ee* (Liang et al. 2013). Furthermore, (*S*)-DFPE was produced on a preparative scale using 100 g/L of DFPA in a 0.5 L reaction system, resulting in a product yield of 90% and *ee* of 99.9% (Liang et al. 2013).

Brivanib, a novel dual inhibitor of the VEGF receptor (VEGFR) and FGF receptor (FGFR) signaling pathways, is currently under clinical evaluation for the treatment of hepatocellular carcinoma (HCC), which is the fifth most common primary neoplasm. (*R*)-1-[4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy]-propan-2-ol ((*R*)-MPO) is an important precursor for the synthesis of brivanib. Codexis provided a series of engineered ketoreductases from ADH-LK and ADH-LB to reduce 1-[4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methyl-pyrrolo[2,1-f][1,2,4]triazin-6-yloxy]-propan-2-one to (*R*)-MPO with high enantioselectivity (Ching et al. 2013).

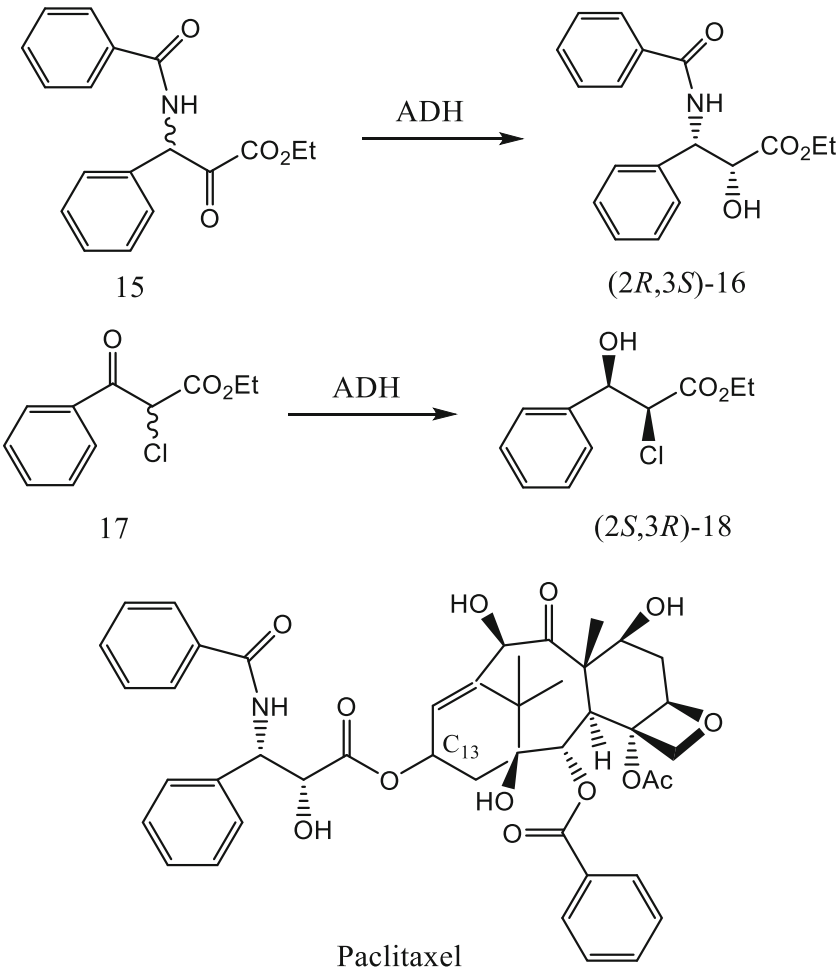

Fig. 6 Bioreduction process for synthesis of taxol side chain intermediates (Applegate et al. 2011; Kearns and Kayser 1994)

Using glucose/glucose dehydrogenase or isopropanol for cofactor recycling, the product was obtained with >99% *ee* at 12 g/L substrate concentration (Ching et al. 2013).

The type 1 insulin-like growth factor receptor (IGF-1R) plays an important role in the establishment and maintenance of the transformed phenotype. It has strong antiapoptotic activity and a significant influence on the control of cell and body size. Downregulation of IGF-1R leads to massive apoptosis of cancer cells, which is an attractive target for cancer therapy. (*S*)-2-chloro-1-(3-chlorophenyl)ethanol ((*S*)-CCPE) is an important intermediate in the synthesis of IGF-1R inhibitors. Hanson et al. (2005) investigated 119 microorganisms for the asymmetric reduction of 2-chloro-1-(3-chlorophenyl)ethanone (CCPEO) and found that whole *H. polymorpha* ATCC 58401 and *Rhodococcus globerulus* ATCC 21505 cells exhibited relatively high enantioselectivity for producing the desired (*S*)-CCPE, with 73.8 and 71.8% *ee*, respectively. Hanson et al. (2005) also found that whole *E. coli* cells containing a recombinant ketoreductase from *H. polymorphato* were able to catalyze the synthesis of (*S*)-CCPE with 100% *ee*. Furthermore, the coexpression of ketoreductase and glucose-6-phosphate dehydrogenase reduced CCPEO to the corresponding (*S*)-CCPE, with 89% yield and 100% *ee* (Hanson et al. 2005). In addition, whole *G. candidum* and *Candida ontarioensis* cells were applied to the asymmetric synthesis of (*R*)-CCPE, with >98% *ee* and >94% yield (Hamada et al. 2001).

Antiemetic drugs

Aprepitant (Emend®) is a neurokinin (NK1) receptor antagonist that is available to prevent chemotherapy-induced nausea and vomiting (CINV) and is used as a relatively new antiemetic drug. (*R*)-1-[3,5-Bis(trifluoromethyl)phenyl]ethanol ((*R*)-BTPE) is a pharmaceutically important alcohol intermediate for the synthesis of aprepitant. Asymmetric reduction of 3,5-bis(trifluoromethyl)acetophenone (BTAP) to (*R*)-BTPE using biocatalysts has been studied (Wang et al. 2014a). Various commercially available ADHs, including HLADH, YADH, BYADH, and *L. kefir* ADH, were tested for the asymmetric reduction of BTAP, of which only *L. kefir* ADH showed an activity with 100% *ee* (Gelo-Pujic et al. 2006). Furthermore, several microorganisms were screened to prepare (*R*)-BTPE, and whole *Penicillium expansum* EBK-9 cells

were identified as the most productive strain, which is able to reduce 3.35 g/L BTAP to (*R*)-BTPE with a 76% yield and >99% *ee* (Kurbanoglu et al. 2009). Whole *Leifsonia xyli* HS0904 and *Trichoderma asperellum* ZJPH0810 cells were subsequently shown to produce (*R*)-BTPE with BTAP concentrations of 70 and 50 mM, respectively. Under the optimized conditions, *L. xyli* HS0904 exhibited a 62% yield and 99.4% *ee*, whereas *T. asperellum* ZJPH0810 exhibited a 93.4% yield and 98% *ee* (Li et al. 2013; Wang et al. 2011b). More recently, a novel strain *Chryseobacterium* sp. CA49 that was capable of reducing BTAP to (*R*)-BTPE was reported, and the substrate concentration could be loaded up to 50 g/L (Liu et al. 2014). After sequencing the *Chryseobacterium* sp. CA49 genome, a recombinant ChKRED20 was identified and shown to synthesize (*R*)-BTPE with a >99% conversion and >99.9% *ee* at a BTAP concentration of 200 g/L using 2-propanol for cofactor regeneration, indicating the great potential for the industrial-scale application of ChKRED20 and production of (*R*)-BTPE (Liu et al. 2014).

Anti-AIDS drugs

Atazanavir, an aza-dipeptide, is a potent human immunodeficiency virus type-1 (HIV-1) protease inhibitor approved for the clinical therapy of acquired immune deficiency syndrome (AIDs). (*1S, 2R*)-[3-Chloro-2-hydroxy-1-(phenylmethyl)propyl]carbamic acid, 1,1-dimethylethyl ester ((*1S,2R*)-CADE) is a key chiral intermediate for atazanavir synthesis. The microbial diastereoselective reduction of (1*S*)-[3-chloro-2-oxo-1-(phenylmethyl)propyl]carbamic acid, 1,1-dimethyl-ethyl ester to (*1S,2R*)-CADE was performed using *Rhodococcus*, *Brevibacterium*, and *Hansenula* strains (Patel et al. 2003). When whole *Rhodococcus erythropolis* SC 13845 cells were used as the biocatalyst, (*1S,2R*)-CADE was obtained with 95% yield, 98.2% *de*, and 99.4% *ee*.

Adrenergic receptor agonists

(*R*)-Denopamine, [(*R*)-(−)-α-(3,4-dimethoxyphenethylaminomethyl)-4-hydroxybenzyl alcohol] and (*R*)-salmeterol, [(*R*)-1,3-benzenedimethanol, 4-hydroxy-α-[((6-(4-phenyl-butoxy)hexyl)amino)methyl], are important β-adrenoreceptor agonists that are approved to treat congestive heart failure. The asymmetric reduction of ketones **19a**–**19e** is an efficient way to obtain the corresponding alcohols (*R*)-**20a**–**20e**, which are key intermediates in the production of (*R*)-denopamine and (*R*)-salmeterol (Fig. 7). The enzyme from *Daucuscarota* root was previously used as a biocatalyst to synthesize compounds (*R*)-**20a** and (*R*)-**20b** with high enantioselectivity (Yadav et al. 2002). Whole *R. rubra* cells were used for the reduction of compounds **19c** and **19d** to obtain compounds (*R*)-**20c** and (*R*)-**20d** with 95% *ee* and 78–80% yield (Goswami et al. 2002). Furthermore, a concise approach was developed for the synthesis of (*R*)-salmeterol by converting compounds **19d** and **19e** to compounds (*R*)-**20d** and (*R*)-**20e** using a novel *Rhodococcus* sp. 1-0130 strain, in which both the aldehyde and ketone groups are reduced (Zhang et al. 2013a).

Other optically pure substituted α-halohydrins also play important roles in the synthesis of β-adrenergic receptor agonists. Whole *S. cerevisiae* CGMCC 2.396, *Aspergillus sydowii* Ce19, and *Pichia minuta* JCM 3622 cells showed high activity and enantioselectivity for the synthesis of (*R*)-α-halohydrins or (*S*)-α-halohydrins (>99% *ee*) (Lin et al. 2009; Rocha et al. 2010; Tokoshima et al. 2013). A substrate-tolerant carbonyl reductase from *Kluyveromyces thermotolerans* (KrtCR) was successfully applied to the asymmetrical reduction of different ketones to corresponding chiral alcohols with 99% *ee* (Xu et al. 2012). Using α-choloacetophenone as a representative substrate, as much as 154 g/L of the substrate was successfully reduced within 12 h, and a yield of 92%, an enantiopurity of >99% *ee*, and a turnover number of 5000 were observed, indicating that KrtCR has great potential in the preparation-scale synthesis of valuable α-halohydrins as versatile chiral synthons (Xu et al. 2012).

(*R*)-Phenylephrine ((*R*)-PE), a selective α_1-adrenergic receptor agonist, is widely used as a substitute for pseudoephedrine and has been approved for the treatment of the common cold, without the side effects caused by other ephedrine adrenergic drugs. Codexis provided a series of engineered ketoreductases from *L. kefir* ADH for the stereoselective reduction of 1-(3-hydroxyphenyl)-2-(methylamino)ethanone (HPMAE) to (*R*)-PE, which exhibited a relative activity that was at least 10-fold higher than the wild-type enzyme and an improved stereoselectivity with >99% *ee* (Alvizo et al. 2012). Furthermore, an NADPH-dependent short-chain dehydrogenase/reductase from *Serratia marcescens* BCRC 10948 was applied to synthesize (*R*)-PE with >99% *ee*. The productivity and yield were 0.57 mmol/h and 51.06%, respectively, at substrate concentration of 10 mM (Peng et al. 2014). In addition, Peng et al. (2014) discovered another short-chain ADH from *Serratia quinivorans* BCRC 14811 for the reduction of HPMAE to generate (*S*)-PE with >99% of *ee*, 86.6% of conversion, and 20.2 mmol/h of productivity.

Biological applications of ADHs to the preparation of fine chemicals

γ-Valerolactone

γ-Valerolactone (GVL) has been identified as a potential intermediate for the production of fuels. Targeted GVL production can be produced from methyl levulinate using ADHs. Díaz-Rodríguez et al. (2013) employed different ADHs from *R. ruber* (ADH-A), *L. brevis* (LBADH), and *Ralstonia* sp.

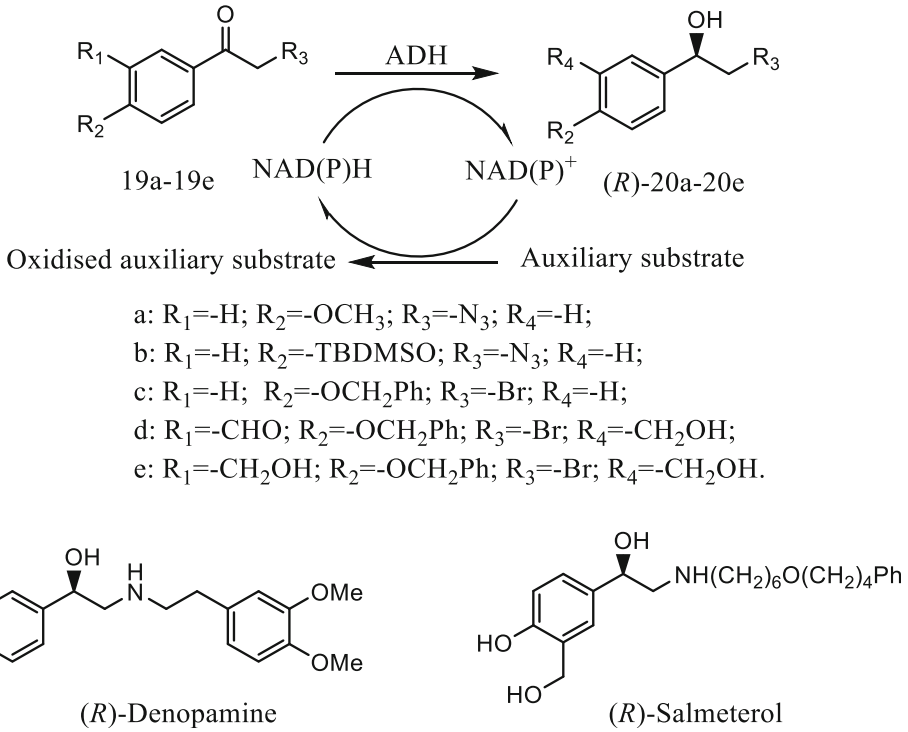

Fig. 7 Bioreduction process for synthesis of (*R*)-denopamine and (*R*)-salmeterol intermediate (Goswami et al. 2002; Yadav et al. 2002; Zhang et al. 2013a)

(RasADH) to synthesize a series of enantiopure hydroxyl esters and lactones, and enantioenriched GVL was obtained with >99% conversion and >99% *ee* using LBADH and ADH-A as biocatalysts. During this process, the cofactor was recycled by the addition of isopropanol (Díaz-Rodríguez et al. 2013).

(2S,3S)-2,3-butanediol

2,3-Butanediol is a promising bulk chemical with extensive industrial applications. ADHs show the capability to reduce diacetyl to acetoin and then to three isomers of 2,3-butanediol, including meso-2,3-butanediol, (*2R,3R*)-2,3-butanediol, and (*2S,3S*)-2,3-butanediol, where optically pure 2,3-butanediol with two vicinal stereogenic centers is used as an important building block for chiral compounds (Ji et al. 2009). Wang et al. (2014b) developed a novel *R. erythropolis* WZ010 strain that was capable of reducing diacetyl to optically pure (*2S,3S*)-2,3-butanediol, in which the purified ADH displayed absolute stereospecificity in the reduction of diacetyl to (*2S,3S*)-2,3-butanediol via (*S*)-acetoin with 98% yield and 100% *ee*. Coupled with the stereoselective oxidation of 1-phenylethanol in the process of preparing (*2S,3S*)-2,3-butanediol, (*R*)-1-phenylethanol was simultaneously produced with 49.4% yield and 99.9% *ee* (Wang et al. 2014c).

Phenylethanols

Optically active phenylethanols and their derivatives are important intermediates in the synthesis of enantiomerically pure pharmaceuticals and fine chemicals. Prochiral acetophenone is usually used as a model substrate for the isolation of microorganisms with ADH activity. Singh et al. (2012) employed whole *Metschnikowia koreensis* cells for the asymmetric reduction of various substituted acetophenones. With 25 mM acetophenone as substrate, 92% conversion and >99% *ee* were obtained. Furthermore, a non-conventional thermotolerant *K. marxianus* cell was shown to be a promising biocatalyst for the production of optically active phenylethanols, and it was used for the bioreduction of different acetophenone derivatives to (*S*)-alcohols (Vitale et al. 2013). In addition, an immobilized short-chain ADH from *T. thermophilus* HB27 was used for the asymmetric reduction of 2,2,2-trifluoroacetophenone to (*S*)-α-(trifluoromethyl)benzyl alcohol with 96% *ee* (Rochamartín et al. 2012), and a purified ADH from *Thermococcus kodakarensis* KOD1 (TkADH) could reduce 2,2,2-trifluoroacetophenone to (*R*)-2,2,2-trifluoro-1-phenyl ethanol with high enantioselectivity (>99.6% *ee*) (Wu et al. 2013).

Conclusion

ADHs are important enzymes that catalyze interconversion between alcohols and aldehydes or ketones under mild conditions. The fundamental research and wide applications of ADHs in the preparation of enantiomerically pure compounds have drawn more attention in recent years. With the development of screening approaches, such as metagenome

technology and genome mining, novel ADHs are expected to be identified and used as promising biocatalysts. The combination of rational and irrational protein design technologies provides a promising strategy to improve the catalytic properties of ADHs for their industrial applications in the production of high-value chemicals and pharmaceuticals.

Acknowledgments The Research Project of the National Natural Science Foundation of China (no. 21672190) and the Major Program for Key Science and Technology of Zhejiang Province of China (no. 2014C03010) are gratefully acknowledged for providing financial support.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

References

Alvizo O, Collier SJ, Hennemann J, Oh SH, Zha W (2012) Ketoreductase polypeptides for the preparation of phenylephrine. European Patent EP 2467473:A2

Applegate GA, Cheloha RW, Nelson DL, Berkowitz DB (2011) A new dehydrogenase from *Clostridium acetobutylicum* for asymmetric synthesis: dynamic reductive kinetic resolution entry into the Taxotère side chain. Chem Commun 47:2420–2422

Broussy S, Cheloha RW, Berkowitz DB (2008) Enantioselective, ketoreductase-based entry into pharmaceutical building blocks: ethanol as tunable nicotinamide reductant. Org Lett 11:305–308

Cai P, An M, Xu L, Xu S, Hao N, Li Y, Guo K, Yan M (2012) Development of a substrate-coupled biocatalytic process driven by an NADPH-dependent sorbose reductase from *Candida albicans* for the asymmetric reduction of ethyl 4-chloro-3-oxobutanoate. Biotechnol Lett 34:2223–2227

Carvalho C C (2016) Whole cell biocatalysts: essential workers from nature to the industry. Microb Biotechnol (http://onlinelibrary.wiley.com/doi/10.1111/1751-7915.12363/epdf)

Chen X, Liu ZQ, Huang JF, Lin CP, Zheng YG (2015) Asymmetric synthesis of optically active methyl-2-benzamido-methyl-3-hydroxy-butyrate by robust short-chain alcohol dehydrogenases from *Burkholderia gladioli*. Chem Commun 51:12328–12331

Chen X, Liu ZQ, Lin CP, Zheng YG (2016) Chemoenzymatic synthesis of (*S*)-duloxetine using carbonyl reductase from *Rhodosporidium toruloides*. Bioorg Chem 65:82–89

Chen YZ, Lin H, Xu XY, Xia SW, Wang LX (2008) Preparation the key intermediate of angiotensin-converting enzyme (ACE) inhibitors: high enantioselective production of ethyl (*R*)-2-hydroxy-4-phenylbutyrate with *Candida boidinii* CIOC21. Adv Synth Catal 350:426–430

Ching C, Gruber JM, Huisman GW, Mundorff E, Newman LM (2013) Ketoreductases and uses thereof. Patent US 8415127:B2

Díaz-Rodríguez A, Borzęcka W, Lavandera I, Gotor V (2013) Stereodivergent preparation of valuable γ- or δ-hydroxy esters and lactones through one-pot cascade or tandem chemoenzymatic protocols. ACS Catal 4:386–393

D'Arrigo P, Pedrocchi-Fantoni G, Servi S (2010) Chemo-enzymatic synthesis of ethyl (*R*)-2-hydroxy-4-phenylbutyrate. Tetrahedron Asymmetry 21:914–918

Davis CS, Grate JH, Gray DR, Gruber JM, Huisman GW, Ma SK, Newman LM, Sheldon R, Wang LA (2010) Enzymatic processes for the production of 4-substituted 3-hydroxybutyric acid derivatives. Patent US 7807423:B2

Erdélyi B, Szabó A, Seres G, Birincsik L, Ivanics J, Szatzker G, Poppe L (2006) Stereoselective production of (*S*)-1-aralkyl- and 1-arylethanols by freshly harvested and lyophilized yeast cells. Tetrahedron Asymmetry 17:268–274

Friest JA, Maezato Y, Broussy S, Blum P, Berkowitz DB (2010) Use of a robust dehydrogenase from an archael hyperthermophile in asymmetric catalysis–dynamic reductive kinetic resolution entry into (*S*)-Profens. J Am Chem Soc 132:5930–5931

Galletti P, Emer E, Gucciardo G, Quintavalla A, Pori M, Giacomini D (2010) Chemoenzymatic synthesis of (2*S*)-2-arylpropanols through a dynamic kinetic resolution of 2-arylpropanals with alcohol dehydrogenases. Org Biomol Chem 8:4117–4123

Gandolfi R, Cesarotti E, Molinari F, Romano D, Rimoldi I (2009) Asymmetric reductions of ethyl 2-(benzamidomethyl)-3-oxobutanoate by yeasts. Tetrahedron Asymmetry 20:411–414

Gelo-Pujic M, Le Guyader F, Schlama T (2006) Microbial and homogenous asymmetric catalysis in the reduction of 1-[3,5-bis(trifluoromethyl)phenyl]ethanone. Tetrahedron Asymmetry 17:2000–2005

Giacomini D, Galletti P, Quintavalla A, Gucciardo G, Paradisi F (2007) Highly efficient asymmetric reduction of arylpropionic aldehydes by horse liver alcohol dehydrogenase through dynamic kinetic resolution. Chem Commun 39:4038–4040

Goldberg S, Guo ZW, Chen S, Goswami A, Patel RN (2008) Synthesis of ethyl-(3*R*,5*S*)-dihydroxy-6-benzyloxyhexanoates via diastereo- and enantioselective microbial reduction: cloning and expression of ketoreductase III from *Acinetobacter* sp. SC 13874. Enzym Microb Technol 43:544–549

Goldberg SL, Nanduri VB, Chu L, Johnston RM, Patel RN (2006) Enantioselective microbial reduction of 6-oxo-8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione: cloning and expression of reductases. Enzym Microb Technol 39:1441–1450

Goswami J, Bezbaruah RL, Goswami A, Borthakur N (2002) A convenient stereoselective synthesis of (*R*)-(−)-denopamine and (*R*)-(−)-salmeterol. Tetrahedron Asymmetry 12:3343–3348

Guo ZW, Chen YJ, Goswami A, Hanson RL, Patel RN (2006) Synthesis of ethyl and t-butyl (3*R*,5*S*)-dihydroxy-6-benzyloxy hexanoates via diastereo- and enantioselective microbial reduction. Tetrahedron Asymmetry 17:1589–1602

Hall M, Bommarius AS (2011) Enantioenriched compounds via enzyme-catalyzed redox reactions. Chem Rev 111:4088–4110

Hamada H, Miura T, Kumobayashi H, Matsuda T, Harada T, Nakamura K (2001) Asymmetric synthesis of (*R*)-2-chloro-1-(m-chlorophenyl)ethanol using acetone powder of *Geotrichum candidum*. Biotechnol Lett 23:1603–1606

Hanson R, Goldberg S, Goswami A, Tully T, Patel R (2005) Purification and cloning of a ketoreductase used for the preparation of chiral alcohols. Adv Synth Catal 347:1073–1080

He J, Mao X, Sun Z, Zheng P, Ni Y, Xu Y (2007) Microbial synthesis of ethyl (*R*)-4,4,4-trifluoro-3-hydroxybutanoate by asymmetric reduction of ethyl 4,4,4-trifluoroacetoacetate in an aqueous-organic solvent biphasic system. Biotech J 2:260–265

Homann MJ, Previte E (1997) Stereoselective microbial reduction of 5-fluorophenyl-5-oxo-pentanoic acid and a phenyloxazolidinone condensation product thereof. Patent US 5618707 A

Isotani K, Kurokawa J, Itoh N (2012) Production of (*R*)-3-quinuclidinol by *E. coli* biocatalysts possessing NADH-dependent 3-quinuclidinone reductase (QNR or bacC) from *Microbacterium*

luteolum and *Leifsonia* alcohol dehydrogenase (LSADH). Int J Mol Sci 13:13542–13553

Itoh N, Isotani K, Nakamura M, Inoue K, Isogai Y, Makino Y (2012) Efficient synthesis of optically pure alcohols by asymmetric hydrogen-transfer biocatalysis: application of engineered enzymes in a 2-propanol–water medium. Appl Microbiol Biotechnol 93: 1075–1085

Ji XJ, Huang H, Zhu JG, Ren LJ, Nie ZK, Du J, Li S (2009) Engineering *Klebsiella oxytoca* for efficient 2,3-butanediol production through insertional inactivation of acetaldehyde dehydrogenase gene. Appl Microbiol Biotechnol 85:1751–1758

Jeong M, Lee YM, Hong SH, Park SY, Yoo IK, Han MJ (2010) Optimization of enantioselective synthesis of methyl (*R*)-2-chloromandelate by whole cells of *Saccharomyces cerevisiae*. Biotechnol Lett 32:1529–1531

Kaliaperumal T, Gummadi SN, Chadha A (2011) Synthesis of both enantiomers of ethyl-4-chloro-3-hydroxbutanoate from a prochiral ketone using *Candida parapsilosis* ATCC 7330. Tetrahedron Asymmetry 22:1548–1552

Kara S, Spickermann D, Weckbecker A, Leggewie C, Arends IW, Hollmann F (2014) Bioreductions catalyzed by an alcohol dehydrogenase in non-aqueous media. ChemCatChem 6:973–976

Kearns J, Kayser MM (1994) Application of yeast-catalyzed reductions to synthesis of (2*R*,3*S*)-phenylisoserine. Tetrahedron Lett 35:2845–2848

Kurbanoglu EB, Zilbeyaz K, Taskin M, Kurbanoglu NI (2009) Total production of (*R*)-3,5-bistrifluoromethylphenyl ethanol by asymmetric reduction of 3,5-bis(trifluoromethyl)-acetophenone in the submerged culture of *Penicillium expansum* isolate. Tetrahedron Asymmetry 20:2759–2763

Li J, Wang P, He JY, Huang J, Tang J (2013) Efficient biocatalytic synthesis of (*R*)-[3,5-bis (trifluoromethyl)phenyl]ethanol by a newly isolated *Trichoderma asperellum* ZJPH0810 using dual cosubstrate: ethanol and glycerol. Appl Microbiol Biotechnol 97:6685–6692

Li N, Ni Y, Sun ZH (2010) Purification and characterization of carbonyl reductase from *Candida krusei* SW 2026 involved in enantioselective reduction of ethyl 2-oxo-4-phenylbutyrate. J Mol Catal B Enzym 66:190–197

Liang J, Borup B, Mitchell V, Mundorff E, Lalonde J, Huisman GW (2012) Ketoreductases for the production of *(S,E)*-methyl 2-(3-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-hroxypropyl) benzoate. Patent US 8088610 B2

Liang J, Jenne SJ, Mundorff E, Ching C, Gruber JM, Krebber A, Huisman GW (2013) Methods of using engineered ketoreductase polypeptides for the stereoselective reduction of acetophenones. Patent US 8512973

Liang J, Mundorff E, Voladri R, Jenne S, Gilson L, Conway A, Krebber A, Wong J, Huisman G, Truesdel S, Lalonde J (2009) Highly enantioselective reduction of a small heterocyclic ketone: biocatalytic reduction of tetrahydrothiophene-3-one to the corresponding (*R*)-alcohol. Org Process Res Dev 14:188–192

Lin H, Chen YZ, Xu XY, Xia SW, Wang LX (2009) Preparation of key intermediates of adrenergic receptor agonists: highly enantioselective production of (*R*)-α-halohydrins with *Saccharomyces cerevisiae* CGMCC 2.396. J Mol Catal B Enzym 57:1–5

Liu Y, Tang TX, Pei XQ, Zhang C, Wu ZL (2014) Identification of ketone reductase *Ch*KRED20 from the genome of *Chryseobacterium* sp. CA49 for highly efficient *anti*-Prelog reduction of 3,5-bis(trifluoromethyl)acetophenone. J Mol Catal B Enzym 102:1–8

Liu ZQ, Ye JJ, Shen ZY, Hong HB, Yan JB, Lin Y, Chen ZX, Zheng YG (2015) Upscale production of ethyl (*S*)-4-chloro-3-hydroxybutanoate by using carbonyl reductase coupled with glucose dehydrogenase in aqueous-organic solvent system. Appl Microbiol Biotechnol 99: 2119–2129

Luo X, Wang YJ, Shen W, Zheng YG (2016) Activity improvement of a *Kluyveromyces lactis* aldo-keto reductase KlAKR via rational design. J Biotechnol 224:20–26

Luo X, Wang YJ, Zheng YG (2015) Cloning and characterization of a NADH-dependent aldo-keto reductase from a newly isolated *Kluyveromyces lactis* XP1461. Enzym Microb Technol 77:68–77

Ma HM, Yang LL, Ni Y, Zhang J, Li CX, Zheng GW, Yang HY, Xu JH (2012) Stereospecific reduction of methyl *o*-chlorobenzoylformate at 300 $g{\cdot}L^{-1}$ without additional cofactor using a carbonyl reductase mined from *Candida glabrata*. Adv Synth Catal 354:1765–1772

Matsuyama A, Yamamoto H, Kawada N, Kobayashi Y (2001) Industrial production of (*R*)-1,3-butanediol by new biocatalysts. J Mol Catal B Enzym 11:513–521

Mundorff E, Vries ED (2013) Polynucleotides encoding recombinant ketoreductase polypeptides. Patent US 8415126 B2

Musa MM, Phillips RS (2011) Recent advances in alcohol dehydrogenase-catalyzed asymmetric production of hydrophobic alcohols. Catal Sci Technol 1:1311–1323

Nealon CM, Musa MM, Patel JM, Phillips RS (2015) Controlling substrate specificity and stereospecificity of alcohol dehydrogenases. ACS Catal 2:2100–2114

Nelson TD, Leblond CR, Frantz DE, Matty L, Mitten JV, Weaver DG, Moore JC, Kim JM, Boyd R, Kim PY, Gbewonyo K, Brower M, Sturr M, McLaughlin K, McMaster DR, Kress MH, McNamara JM, Dolling UH (2004) Stereoselective synthesis of a potent thrombin inhibitor by a novel P2-P3 lactone ring opening. J Org Chem 69: 3620–3627

Ni Y, Li CX, Zhang J, Shen ND, Bornscheuer UT, Xu JH (2011) Efficient reduction of ethyl 2-oxo-4-phenylbutyrate at 620 $g{\cdot}L^{-1}$ by a bacterial reductase with broad substrate spectrum. Adv Synth Catal 353: 1213–1217

Ni Y, Pan J, Ma HM, Li CX, Zhang J, Zheng GW, Xu JH (2012) Bioreduction of methyl *o*-chlorobenzoylformate at 500 g/L without external cofactors for efficient production of enantiopure clopidogrel intermediate. Tetrahedron Lett 53:4715–4717

Ni Y, Su YN, Li HD, Zhou JY, Sun ZH (2013) Scalable biocatalytic synthesis of optically pure ethyl (*R*)-2-hydroxy-4-phenylbutyrate using a recombinant *E. coli* with high catalyst yield. J Biotechnol 168:493–498

Noyori R, Ikeda T, Ohkuma T, Widhalm M, Kitamura M, Takaya H, Akutagawa S, Sayo N, Saito T, Taketomi T, Kumobayashi H (1989) Stereoselective hydrogenation via dynamic kinetic resolution. J Am Chem Soc 111:9134–9135

Oliveira SSDS, Dias LRS, Barbosa NC, Bello ML, Novaes FJM, Neto FRDA, Fiaux SB (2013) Enantioselective bioreduction of ethyl 4,4,4-trihalide-3-oxobutanoate by *Kluyveromyces marxianus*. Tetrahedron Lett 54:3067–3070

Patel R, Chu L, Nanduri V, Li J, Kotnis A, Parker W (2005) Enantioselective microbial reduction of 6-oxo-8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione. Tetrahedron Asymmetry 16:2778–2783

Patel RN (2013) Biocatalytic synthesis of chiral alcohols and amino acids for development of pharmaceuticals. Biomolecules 3:741–777

Patel RN, Banerjee A, Howell JM, Mcnamee CG, Brzozowski D, Nanduri V, Thottathil JK, Szarka LOJ (1995) Stereoselective microbial reduction of 2-keto-3-(*N*-benzoylamino)-3-phenyl propionic acid ethyl ester. Ann N Y Acad Sci 750:166–174

Patel RN, Chu L, Mueller R (2003) Diastereoselective microbial reduction of (*S*)-[3-chloro-2-oxo-1-(phenylmethyl)propyl]carbamic acid, 1,1-dimethylethyl ester. Tetrahedron Asymmetry 14:3105–3109

Patel RN, Mcnamee CG, Banerjee A, Howell JM, Robison RS, Szarka LJ (1992) Stereoselective reduction of β-keto esters by *Geotrich candidum*. Enzym Microb Technol 14:731–738

Peng GJ, Kuan YC, Chou HY, Fu TK, Lin JS, Hsu WH, Yang MT (2014) Stereoselective synthesis of (*R*)-phenylephrine using recombinant *Escherichia coli* cells expressing a novel short-chain dehydrogenase/reductase gene from *Serratia marcescens* BCRC 10948. J Biotechnol 170:6–9

Pennacchio A, Giordano A, Rossi M, Raia CA (2011) Asymmetric reduction of α-keto esters with *Thermus thermophilus* NADH-dependent carbonyl reductase using glucose dehydrogenase and alcohol dehydrogenase for cofactor regeneration. Eur J Org Chem 2011:4361–4366

Qian XL, Pan J, Shen ND, Ju X, Zhang J, Xu JH (2014) Efficient production of ethyl (*R*)-2-hydroxy-4-phenylbutyrate using a cost-effective reductase expressed in *Pichia pastoris*. Biochem Eng J 91:72–77

Quaglia D, Pori M, Galletti P, Emer E, Paradisi F, Giacomini D (2013) His-tagged horse liver alcohol dehydrogenase: immobilization and application in the bio-based enantioselective synthesis of (*S*)-arylpropanols. Process Biochem 48:810–818

Rimoldi I, Cesarotti E, Zerla D, Molinari F, Albanese D, Castellano C, Gandolfi R (2011) 3-(Hydroxy(phenyl)methyl)azetidin-2-ones obtained via catalytic asymmetric hydrogenation or by biotransformation. Tetrahedron Asymmetry 22:597–602

Roberge C, King A, Pecore V, Greasham R, Chartrain M (1996) Asymmetric bioreduction of a keto ester to its corresponding (*S*)-hydroxy ester by *Microbacterium* sp. MB 5614. J Ferment Bioeng 81:530–533

Rochamartín J, Vega D, Bolivar JM, Hidalgo A, Berenguer J, Guisán JM, Lopez-Gallego F (2012) Characterization and further stabilization of a new anti-Prelog specific alcohol dehydrogenase from *Thermus thermophilus* HB27 for asymmetric reduction of carbonyl compounds. Bioresour Technol 103:343–350

Rocha LC, Ferreira HV, Pimenta EF, Berlinck RG, Rezende MO, Landgraf MD, Seleghim MHR, Sette LD, Porto ALM (2010) Biotransformation of α-bromoacetophenones by the marine fungus *Aspergillus sydowii*. Mar Biotechnol 12:552–557

Savile C, Gruber JM, Mundorff E, Huisman G, Collier SJ (2014) Ketoreductase polypeptides for the production of a 3-aryl-3-hydroxypropanamine from a 3-aryl-3-ketopropanamine. Patent US 8673607B2

Sheng B, Zheng Z, Lv M, Zhang H, Qin T, Gao C, Ma CQ, Xu P (2014) Efficient production of (*R*)-2-hydroxy-4-phenylbutyric acid by using a coupled reconstructed d-lactate dehydrogenase and formate dehydrogenase system. PLoS One 9:e104204

Shimoda K, Kubota N, Hamada H, Kobayashi T, Hamada H, Shafi SM, Nakajima N (2009) Production of (2*R*,3*S*)-2-benzamidomethyl-3-hydroxybutanoates by immobilized plant cells of *Parthenocissus tricuspidata*. Biochem Insights 2:5–7

Simon RC, Busto E, Richter N, Belaj F, Kroutil W (2014) Chemoenzymatic synthesis of enantiomerically pure syn-configured 1-aryl-3-methylisochroman derivatives. Eur J Org Chem 2014:111–121

Singh A, Basit A, Banerjee UC (2009) Burkholderia cenocepacia: a new biocatalyst for efficient bioreduction of ezetimibe intermediate. J Ind Microbiol Biot 36:1369–1374

Singh A, Chisti Y, Banerjee UC (2012) Stereoselective biocatalytic hydride transfer to substituted acetophenones by the yeast *Metschnikowia koreensis*. Process Biochem 47:2398–2404

Soni P, Kansal H, Banerjee UC (2008) Optimization of process parameters for the production of carbonyl reductase by *Candida viswanathii* in a laboratory-scale fermentor. J Ind Microbiol Biotechnol 35:167–173

Su YN, Ni Y, Wang JC, Xu ZH, Sun ZH (2012) Two-enzyme coexpressed recombinant strain for asymmetric synthesis of ethyl (*R*)-2-hydroxy-4-phenylbutyrate. Chinese J Catal 33:1650–1660

Sun XY, Shi HB, Bi HX, Ou ZM (2013) Bioconversion process for synthesis of *tert*-butyl (3*R*,5*S*)-6-chloro-3,5-dihydroxyhexanoate using liquid-core immobilized *Saccharomyces cerevisiae* CGMCC no 2233. Korean J Chem Eng 30:166–171

Tang CG, Lin H, Zhang C, Liu ZQ, Yang T, Wu ZL (2011) Highly enantioselective bioreduction of *N*-methyl-3-oxo-3-(thiophen-2-yl) propanamide for the production of (*S*)-duloxetine. Biotechnol Lett 33:1435–1440

Tixidre A, Zard L, Rossey G, Bourbon A (1998) Process for the resolution of ethyl 4, 4, 4-trifluoro-3 (*R*)-hydroxybutanoate using lipase from *candida*. Patent US 5716841 A.

Tokoshima D, Hanaya K, Shoji M, Sugai T (2013) Whole-cell yeast-mediated preparation of (*R*)-2-chloro-1-(3-nitrophenyl)ethanol as a synthetic precursor for (*R*)-phenylephrine. J Mol Catal B Enzym 97: 95–99

Tsutsumi K, Katayama T, Utsumi N, Murata K, Arai N, Kurono N, Ohkuma T (2009) Practical asymmetric hydrogenation of 3-quinuclidinone catalyzed by the xylSkewphos/PICA− ruthenium (II) complex. Org Process Res Dev 13:625–628

Uzura A, Nomoto F, Sakoda A, Nishimoto Y, Kataoka M, Shimizu S (2009) Stereoselective synthesis of (*R*)-3-quinuclidinol through asymmetric reduction of 3-quinuclidinone with 3-quinuclidinone reductase of *Rhodotorula rubra*. Appl Microbiol Biotechnol 83: 617–626

Vitale P, D'Introno C, Perna FM (2013) *Kluyveromyces marxianus* CBS 6556 growing cells as a new biocatalyst in the asymmetric reduction of substituted acetophenones. Tetrahedron Asymmetry 44:389–394

Volkmann RA, Kelbaugh PR, Nason DM, Jasys VJ (1992) 2-Thioalkyl penems: an efficient synthesis of sulopenem, a (5*R*,6*S*)-6-(1(*R*)-hydroxyethyl)-2-[(cis-1-oxo-3-thiolanyl)thio]-2-penem antibacterial. J Org Chem 57:4352–4361

Wang LJ, Li CX, Ni Y, Zhang J, Liu X, Xu JH (2011a) Highly efficient synthesis of chiral alcohols with a novel NADH-dependent reductase from *Streptomyces coelicolor*. Bioresour Technol 102:7023–7028

Wang P, Cai JB, Ouyang Q, He JY, Su HZ (2011b) Asymmetric biocatalytic reduction of 3,5-bis (trifluoromethyl) acetophenone to (*1R*)-[3,5-bis (trifluoromethyl) phenyl] ethanol using whole cells of newly isolated *Leifsonia xyli* HS0904. Appl Microbiol Biotechnol 90: 1897–1904

Wang Y, Li JJ, Wu QQ, Zhu DM (2013a) Microbial stereospecific reduction of 3-quinuclidinone with newly isolated *Nocardia* sp. and *Rhodococcus erythropolis*. J Mol Catal B Enzym 88:14–19

Wang Y, Wang LQ, He YC, Zhu J, Qing Q, Wang MH (2013b) Biosynthesis of ethyl (*R*)-2-hydroxy-4-phenylbutyrate catalyzed by immobilized *Rhodotorula mucilaginosa* CCZU-G5 in biphasic system. Chem Ind Eng Prog 32:661–665

Wang NQ, Sun J, Huang J, Wang P (2014a) Cloning, expression, and directed evolution of carbonyl reductase from *Leifsonia xyli* HS0904 with enhanced catalytic efficiency. Appl Microbiol Biotechnol 98: 8591–8601

Wang Z, Song QQ, Yu ML, Wang YF, Xiong B, Zhang YJ, Zheng JY, Ying XX (2014b) Characterization of a stereospecific acetoin(diacetyl) reductase from *Rhodococcus erythropolis* WZ010 and its application for the synthesis of (2*S*,3*S*)-2,3-butanediol. Appl Microbiol Biotechnol 98:641–650

Wang Y, Tao F, Xu P (2014c) Glycerol dehydrogenase plays a dual role in glycerol metabolism and 2,3-butanediol formation in *Klebsiella pneumoniae*. J Biol Chem 289:6080–6090

Wang YJ, Liu XQ, Luo X, Liu ZQ, Zheng YG (2015) Cloning, expression and enzymatic characterization of an aldo-keto reductase from *Candida albicans* XP1463. J Mol Catal B Enzym 122:44–50

Wang S, Meng X, Zhou H, Liu Y, Secundo F, Liu Y (2016) Enzyme stability and activity in non-aqueous reaction systems: a mini review. Catalysts 6:32

Wu XR, Liu N, He YM, Chen YJ (2009) Cloning, expression, and characterization of a novel diketoreductase from *Acinetobacter baylyi*. Acta Bioch Bioph Sin 41:163–170

Wu X, Chen C, Liu N, Chen Y (2011) Preparation of ethyl 3*R*,5*S*-6-(benzyloxy)-3,5-dihydroxy-hexanoate by recombinant diketoreductase in a biphasic system. Bioresour Technol 102: 3649–3652

Wu X, Zhang C, Orita I, Imanaka T, Fukui T, Xing XH (2013) Thermostable alcohol dehydrogenase from *Thermococcus kodakarensis* KOD1 for enantioselective bioconversion of aromatic secondary alcohols. Appl Environ Microbiol 79:2209–2217

Xia SW, Chen YZ, Junrui Z, Xu HM (2013) Enzyme-catalyzed asymmetric synthesis of optically active (*R*)- and (*S*)-ethyl-4-phenyl-4-hydroxybutyrate with microbial cells. Biocatal Biotransform 31:66–70

Xu GC, Yu HL, Zhang XY, Xu JH (2012) Access to optically active aryl halohydrins using a substrate-tolerant carbonyl reductase discovered from *Kluyveromyces thermotolerans*. ACS Catal 2:2566–2571

Xu YP, Guan YH, Yu HL, Ni Y, Ma BD, Xu JH (2014) Improved *o*-chlorobenzoylformate bioreduction by stabilizing aldo-keto reductase YtbE with additives. J Mol Catal B Enzym 104:108–114

Yadav JS, Reddy PT, Nanda S, Rao AB (2002) Stereoselective synthesis of (*R*)-(−)-denopamine, (*R*)-(−)-tembamide and (*R*)-(−)-aegeline via asymmetric reduction of azidoketones by *Daucus carota* in aqueous medium. Tetrahedron Asymmetry 12:3381–3385

Yamamoto H, Matsuyama A, Kobayashi Y (2002) Synthesis of (*R*)-1, 3-butanediol by enantioselective oxidation using whole recombinant *Escherichia coli* cells expressing (*S*)-specific secondary alcohol dehydrogenase. Biosci Biotechnol Biochem 66:925–927

Yasohara Y, Yano M, Kawano S, Kizaki N (2009) Method for producing optically active 2-(n-substitued aminomethyl)-3-hydroxybutyric acid ester. Patent US 20090104671A1

Ye Q, Ouyang P, Ying H (2011) A review—biosynthesis of optically pure ethyl (*S*)-4-chloro-3-hydroxybutanoate ester: recent advances and future perspectives. Appl Microbiol Biotechnol 89:513–522

You ZY, Liu ZQ, Zheng YG (2014) Characterization of a newly synthesized carbonyl reductase and construction of a biocatalytic process for the synthesis of ethyl (*S*)-4-chloro-3-hydroxybutanoate with high space-time yield. Appl Microbiol Biotechnol 98:1671–1680

Zhang J, Witholt B, Li Z (2006) Efficient NADPH recycling in enantioselective bioreduction of a ketone with permeabilized cells of a microorganism containing a ketoreductase and a glucose 6-phosphate dehydrogenase. Adv Synth Catal 348:429–433

Zhang R, Ren J, Wang Y, Wu QQ, Wang M, Zhu DM (2013a) Isolation and characterization of a novel *Rhodococcus* strain with switchable carbonyl reductase and *para*-acetylphenol hydroxylase activities. J Ind Microbiol Biotechnol 40:11–20

Zhang R, Xu Y, Xiao R (2015) Redesigning alcohol dehydrogenases/reductases for more efficient biosynthesis of enantiopure isomers. Biotechnol Adv 33:1671–1684

Zhang W, Ni Y, Sun ZH, Zheng P, Lin WQ, Zhu P, Ju N (2009) Biocatalytic synthesis of ethyl (*R*)-2-hydroxy-4-phenylbutyrate with *Candida krusei* SW2026: a practical process for high enantiopurity and product titer. Process Biochem 44:1270–1275

Zhang WX, Xu GC, Huang L, Pan J, Yu HL, Xu JH (2013b) Highly efficient synthesis of (*R*)-3-quinuclidinol in a space–time yield of 916 g L^{-1} d^{-1} using a new bacterial reductase *Ar*QR. Org Lett 15:4917–4919

Zheng RC, Ge Z, Qiu ZK, Wang YS, Zheng YG (2012) Asymmetric synthesis of (*R*)-1,3-butanediol from 4-hydroxy-2-butanone by a newly isolated strain *Candida krusei* ZJB-09162. Appl Microbiol Biotechnol 94:969–976