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'Green' synthesis of important pharmaceutical building blocks: enzymatic access to enantiomerically pure α-chloroalcohols

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Abstract—Thirty one recombinant ketoreductase enzymes were screened for the reduction of six α-chloroketones, the precursors of pharmaceutically valuable α-chloroalcohols. Several highly active and enantioselective ketoreductases were found and their applications to the synthesis of both enantiomers of these α-chloroalcohols were demonstrated on a preparative scale. This offers a convenient, 'green' access to this type of important pharmaceutical building blocks.

1. Introduction

Chiral halohydrins are valuable synthetic intermediates for the preparation of a wide range of biologically interesting compounds. 2-Chloro-1-phenylethanol **1**, 2-chloro-1-(3′-chlorophenyl)-ethanol **2**, 2-chloro-1-(4′-chlorophenyl)ethanol **3**, 2-chloro-1-(3′,4′-dichlorophenyl)ethanol **4**, 2-chloro-1-(4′-nitrophenyl)ethanol **5**, and 2-chloro-1-(4′-methanesulfonamidophenyl)ethanol **6** (Fig. 1) are particularly interesting as synthons for the preparation of a large group of anti-depressants and α- and β-adrenergic drugs.[1–6] For example, 2-chloro-1-phenylethanol **1** is the precursor for the synthesis of fluoxetine, tomoxetine, and nisoxetine,[1] while 2-chloro-1-(3′-chlorophenyl)ethanol **2** has been used to synthesize AJ-9677, a potent and selective β_3 adrenergic receptor agonist (Fig. 1).[2a]

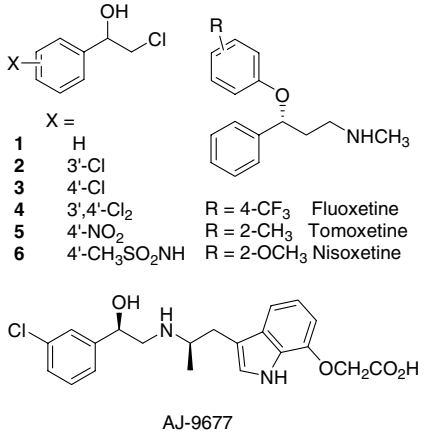

Figure 1. Structures of α-chloroalcohols **1**–**6** and four drugs.

Amongst the various approaches to chiral chlorohydrins, a straightforward process is the asymmetric reduction of prochiral halomethyl ketones, which can be achieved chemically and biocatalytically.[7,8] With ever-increasing environmental concerns, the development of 'green methods' to produce fine chemicals are highly desirable. Biocatalysis accommodates several of the 12 principles of green chemistry defined by Anastas and Warner,[9] hence studies on the biocatalytic reduction of prochiral ketones to the corresponding enantiomerically pure alcohols have been developed rapidly over the last decade.[10]

Biocatalytic reductions can be carried out using either whole cell systems[11] or isolated ketoreductases.[12] For the biocatalytic transformation of α-chloroacetophenones to the corresponding chiral α-chloroalcohols, most of the reported methods involve whole cell reactions.[8,10c] However, the use of isolated enzymes is advantageous because undesirable enantiomer formation mediated by contaminating ketoreductases is minimized,[13] the enzymes can be stored and used as normal chemical reagents and no microbiological knowledge is required. In this regard, we have recently developed a

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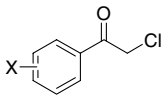

X =

7	H
8	3'-Cl
9	4'-Cl
10	3',4'-Cl_2
11	4'-NO_2
12	4'-CH_3SO_2NH

Figure 2. Structures of α-chloroketones **7**–**12**.

ketoreductase tool-box of 31 recombinant ketoreductase enzymes by genome mining and protein engineering, and have shown that these isolated recombinant enzymes efficiently catalyze the enantioselective reduction of substituted aryl ketones and β-ketoesters.[14] To explore further the application of our isolated recombinant ketoreductase collection, six α-chloroketones **7**–**12** were screened with our enzyme collection (Fig. 2). The enantioselectivities of 12 selected ketoreductases with high activities were then studied. It was found that both enantiomers of the above-mentioned important pharmaceutical building blocks **1**–**6** could be obtained in enantiomerically pure form and good to excellent isolated yields.

2. Results and discussion

The initial reaction rates of α-chloroketones **7**–**11** with the ketoreductase enzymes **KRED101**–**131** were determined by spectrophotometrically measuring the oxidation of NADPH at 340 nm at room temperature. For 4-chloroacetylphenylmethanesulfonamide **12**, the strong absorbance at 340 nm prevented the measurement of reaction rate by this method. As shown in Table 1, **KRED101**, **107**, **111**, **112**, **113**, **114**, **115**, **118**, **121**, **123**, **130**, and **131** were efficient catalysts in the reduction of all the tested α-chloroketones (the data for the less active enzymes are not presented). For most of these 12 ketoreductases, *meta*-chlorinated α-chloroketone **8** showed a higher activity than the unsubstituted one **7**, while *para*-chloro substituent **9** decreased the activity. An exception was **KRED107**. Seven out of twelve ketoreductases **KRED101**, **111**, **112**, **113**, **114**, **115**, and **118** were less active towards the reductions of 3′,4′-dichloro- and 4′-nitro-α-chloroacetophenones **10** and **11** than the unsubstituted counterpart **7**, and the other enzymes showed a reverse tendency.

Table 1. Specific activity of ketoreductase-catalyzed reduction of α-chloroketones **7**–**11**[a]

KRED	**7** (4′-H)	**8** (3′-Cl)	**9** (4′-Cl)	**10** (3′,4′-Cl_2)	**11** (4′-NO_2)
101	1147	1359	523	273	674
107	25.0	20.9	91.1	75.3	106
111	484	503	128	262	142
112	1424	1580	388	396	1015
113	1628	1684	331	513	1397
114	390	392	61.8	88.6	271
115	1095	1466	715	348	531
118	327	365	61	211	276
121	95.7	97.6	15.7	116	104
123	104	167	73.4	170	162
130	148	257	163	299	161
131	17.2	94.6	7.5	82.7	90.4

[a] The specific activity was defined as nmol min^{-1} mg^{-1}.

The enantioselectivity of ketoreductases **KRED101**, **107**, **111**, **112**, **113**, **114**, **115**, **118**, **121**, **123**, **130**, and **131** for the reduction of α-chloroketones **7**–**12** were evaluated using the NADPH recycle system of D-glucose dehydrogenase and D-glucose. The results are presented in Table 2. As shown in Table 2, **KRED101**, **107**, **112**, **113**, **130**, and **131** showed excellent enantioselectivity for the reduction of all the tested α-chloroketones **7**–**12**, and the substituents on the benzene ring of the ketones exerted minimal effect on their enantioselectivity. The reductions catalyzed by **KRED101**, **107**, **112**, or **113** gave (*S*)-chloroalcohols, while (*R*)-configuration products were obtained using **KRED 130** or **131** as catalyst. The enantioselectivities of the other ketoreductase enzymes were greatly affected by the substituents of substrates, and the substituent even reverted the absolute configuration of the major product alcohol. This was exemplified by the **KRED118**-catalyzed reduction of **7** and **8**, in which the (*R*)-enantiomer was produced as the major product with modest enantioselectivity, while the (*S*)-enantiomers were obtained for other α-chloroketones with up to 98% ee.

Good preparative applicability requires high activity and enantioselectivity. Tables 1 and 2 show that, for all the tested α-chloroketones, both enantiomers of the product alcohols could be obtained in greater than 99% enantiomeric excess via the reduction catalyzed by at least one enzyme in the ketoreductase tool box. This was then further tested on a 1 mmol scale with selected enzymes, allowing ready isolation and characterization of the products. The isolated yield and enantiomeric excess (ee) for the preparative scale reactions are summarized in Table 3. The (*R*)-enantiomers of α-chloroalcohols **1**–**6** were prepared with **KRED130** in good to excellent yields (76–99%) and essentially enantiomerically pure form, while the enantiomerically pure (*S*)-enantiomers were obtained in high isolated yields (69–97%) using **KRED112**, **107**, or **113**. It is clearly demonstrated that our ketoreductase collection is useful and provides a 'green' alternative access to enantiomerically pure α-chloro alcohols of pharmaceutical importance.

3. Experimental

The chiral GC analysis was performed on a Hewlett Packard 5890 series II plus gas chromatograph equipped with autosampler, EPC, split/splitless injector, FID detector and 25 m × 0.25 mm CP-Chirasil-Dex CB chiral capillary column. The chiral HPLC analysis was performed on a Water prep4000 with UV detector and (*S*,*S*)-Whelk-O1 (4.6 × 25 cm) chiral column from Regis Technologies, Inc. All the ketoreductases were purified recombinant enzymes, which were developed by genome mining and protein engineering, and are commercially available from BioCatalytics, Inc. α-Chloroketones were