Enhanced Production of (S)-2-arylpropionic Acids by Protein Engineering and Whole-Cell Catalysis

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Esterases are important biocatalysts for chemical synthesis. Several bHSL family esterases have been used to prepare (S)-2-arylpropionic acids with stronger anti-inflammatory effects via kinetic resolution. Here, we presented the discovery of key residues that controlled the enantioselectivity of bHSL family esterases to ethyl 2-arylpropionates, through careful analysis of the structural information and molecular docking. A new bHSL family esterase, Est924, was identified as a promising catalyst for kinetic resolution of racemic ethyl 2-arylpropionates with slight (R)-stereopreference. Using Est924 as the starting enzyme, protein engineering was conducted at hotspots, and the substitution of A203 was proved to enhance the enantioselectivity. The stereopreference of the mutant M1 (A203W) was inverted to ethyl (S)-2-arylpropionates, and this stereopreference was further improved in variant M3 (I202F/A203W/G208F). In addition, the optimal variant, M3, was also suitable for the resolution of ibuprofen ethyl ester and ketoprofen ethyl ester, and their efficient (S)-isomers were synthesized. Next, the whole-cell catalyst harboring M3 was used to prepare (S)-ketoprofen. (S)-ketoprofen with 86%ee was produced by whole-cell catalyst with a single freeze-thaw cycle, and the cells could be reused for at least five cycles. Our results suggested that Est924 variants could kinetically resolve economically important racemates for industrial production and further offer the opportunity for the rational design of enzyme enantioselectivity. Moreover, it is an economical process to prepare optically pure (S)-ketoprofen and (S)-naproxen by using an engineered strain harboring M3 as the catalyst.

Keywords: bHSL family esterase, NSAIDs, enantioselectivity, rational design, whole-cell catalysis

BACKGROUND

Chirality is one of the essential properties of natural substances, and enantiomers are ubiquitous in nature. Different enantiomers of a biologically active molecule generally have different physiological properties, such as different degrees or different types of biological activities, or even opposite bioactivities or toxic side effects (Lough et al., 2012). Non-steroidal anti-inflammatory

Abbreviations: ee, enantiomeric excess; IPTG, isopropyl-β-d-thiogalactopyranoside; NSAIDs, non-steroidal anti-inflammatory drugs.
drugs (NSAIDs) are the world's most used drugs with anti-inflammatory, analgesic, and antipyretic effects. 2-arylpropionate drugs (known as profen drugs) are a very important class of chiral drugs in NSAIDs, including ibuprofen, naproxen, ketoprofen, flurbiprofen, fenoprofen, and loxoprofen, among others. Profen drugs contain a stereogenic center at the carbon alpha of the carboxyl function and therefore racemic (R)- and (S)-enantiomers which are equivalent in mass. A large number of pharmacological studies on the relative activities of the two enantiomers showed that (S)-enantiomers have significantly higher clinical effects, and dexketoprofen, (S) of the two enantiomers showed that (S)-enantiomers have several NSAIDs drugs, including (S)-ibuprofen, (S)-naproxen and dexketoprofen. Compared to the purified enzyme and immobilized enzyme, whole-cell catalyst was more readily prepared, and cell envelopes can be beneficial to stabilize enzymes. Whole-cell catalysis is widely applied as an alternative to conventional chemical methods for pharmaceutical synthesis (Lin and Tao, 2017). Through a simple freeze-thaw process, the cells were highly active and readily usable for the synthesis of (S)-ketoprofen and (S)-naproxen. Our results provide the molecular basis of the enantioselectivity of bHSL family esterases against ethyl (S)-2-arylpropionates, indicating a breakthrough in the development of esterases.

MATERIALS AND METHODS

Construction of the Designed Mutants

Selected residues of Est924 were mutated by site mutagenesis using primers in Supplementary Table 1 with wild-type Est924 plasmid as a template. The introduction of single site mutations into Est924 was constructed using the QuickChange kit (Stratagene, CA, United States) by following the manufacturer’s instructions. The PCR product was incubated with DpnI (New England Biolabs, MA, United States) at 37°C for 1 h to degrade the parental template. Variants were transformed into Escherichia Coli BL21 (DE3) competent cells. Since naproxen is sold as the (S)-enantiomer, the recombinants were picked up and inoculated on lysogeny broth (LB) solid media containing 10 mM racemic naproxen ethyl ester and then cultured at 37°C for 12 h. We picked recombinants with translucent zones and those with transparent zones smaller than Est924. The standard expression and purification conditions were followed to obtain mutant enzymes. The racemic naproxen ethyl ester was subsequently used as substrate and the enantioselectic excess (ee) of products was analyzed by HPLC. The extracted product was purified by flash column chromatography with petroleum ether and ethyl acetate to separate acid and ester. The acid was esterified by the previously described method (Jin et al., 2003) and the ee of the product was analyzed using suitable column with the chiral stationary phase. (Supplementary Table 2) mounted on HPLC (Agilent, CA, United States) and UV detector at 214 nm (Godinho et al., 2012). The enantioselectivity of the esterase was expressed as the enantiomeric ratio (E) and calculation of E-value was performed using the method of Kim et al. (2017). Finally, the genes of the enzymes with increased enantioselectivity were verified by DNA sequencing (Sangon Biotech, Shanghai, China).
Expression and Purification of Est924 and Variants

A single colony was picked up and grown in LB supplemented with kanamycin (50 µg/mL). The overnight culture was used to inoculate main cultures (800 mL LB medium containing 50 µg/mL kanamycin) to an initial OD600 of 0.05. Cells were grown at 37°C to an OD600 of 0.6, and IPTG was added to a final concentration of 0.1 mM. The cultures were incubated at 30°C for an additional 10 h, and cells were harvested by centrifugation at 10,800 g for 20 min. Cell pellets were washed with a NaCl solution (0.9%, w/v) and frozen at −20°C for later purification.

The cell pellets were thawed on ice, resuspended in 10 ml lysis buffer (50 mM phosphate buffer containing 300 mM NaCl and 10 mM imidazole, pH 8.0) per gram of cell pellet and disrupted by sonication (Sonicator QSonica Q500 Ultra Sonicator) at 0°C. Cell debris was removed by centrifugation at 18,000 g for 30 min at 4°C. The supernatant was filtered through a 0.25 µm PVDF filter and was loaded onto a Ni-NTA column (Thermo scientific) equilibrated with lysis buffer. The column was then washed with six column volumes of wash buffer (50 mM phosphate buffer containing 300 mM NaCl and 20 mM imidazole, pH 8.0) after loading the filtered lysate. Finally, the protein was eluted with elution buffer (50 mM phosphate buffer containing 300 mM NaCl and 250 mM imidazole), and fractions containing target enzyme (determined by SDS-PAGE) were pooled and dialyzed at 4°C against Tris–HCl buffer (20 mM, pH 7.5). Protein expression and purity were assessed by SDS-PAGE (Supplementary Figure 1).

Enzymatic Activity Assay

Esterase activity was measured using the standard assay: 50 µg purified esterase was reacted with 10 mM racemic ethyl 2-arylpropionates in 200 mM Tris–HCl buffer (pH 8.0) with 5% DMSO for 30 min at 30°C. The reaction was terminated by adjusting the pH to 2 using 6 M HCl and extracted with ethyl acetate. The organic layers were dried over anhydrous Na2SO4 and evaporated under vacuum. Conversion ratio was measured using NMR. The ee of products was analyzed by HPLC as described above.

Structure Modeling and Molecular Docking

Homology modeling of Est924, and M3 (I202F/A203W/G208F) were performed using the SWISS-MODEL online system using esterase EstD11 (PDB id: 7AT3) as a template (sequence similarity: 49%) (Waterhouse et al., 2018; Miguel-Ruano et al., 2021). The structure of SsoEst1 was simulated in the same manner using 5LK6 as the template (sequence similarity 91.78%). The final models were validated by PROCHECK and Verify 3D (Laskowski et al., 1996). Pymol was employed to visualize and analyze the 3D structure of Est924 and its mutants (DeLano, 2002).

Bioconversion Conditions

After induction, cells were collected by centrifugation at 8,000 × g for 10 min, washed twice with 0.9% brine, and resuspended in the reaction mixture containing 50 mM potassium phosphate buffer (pH 8.0), 10 mM ketoprofen ethyl ester, and 5% DMSO to form cell suspension (OD600 nm = 3) with a total volume of 10 mL (in a 50 mL shake flask). The bioconversion reactions were performed at 30°C and 200 rpm. At different time points,

![FIGURE 1](https://swissmodel.expasy.org)

FIGURE 1 | Substrate specificity and enantioselectivity of carboxylesterase Est924 for ethyl 2-arylpropionates.
Table 1 | Hydrolytic activity of esterase Est924 for ethyl 2-arylpropionates.

| Entry | Substrate | Conversion (%)\(^{[a]}\) | Specific activity (mM/mg/min) | ee%\(^{[b]}\) |
|-------|-----------|--------------------------|-------------------------------|-------------|
| 1     | 1a        | 95                       | 1.5                           | 20%(R)      |
| 2     | (S)−2a    | 90                       | 3.3                           | N.D.        |
| 3     | (R)−2a    | 95                       | 3.5                           | N.D.        |
| 4     | 3a        | 97                       | 3.5                           | 5%(R)       |
| 5     | 4a        | 85                       | 2.7                           | N.D.        |
| 6     | 5a        | 90                       | 3.1                           | N.D.        |
| 7     | 6a        | 82                       | 2.3                           | N.D.        |
| 8     | 7a        | 82                       | 2.4                           | N.D.        |
| 9     | 8a        | 90                       | 2.7                           | 5%(R)       |

\(^{[a]}\) Conversions were determined by NMR.
\(^{[b]}\) Enantiomeric excess values were determined by chiral HPLC. N.D., not determined.

100 µL of sample was taken and mixed with 4 µL 6 M HCl and extracted with EtOAc. The resulting supernatant was centrifuged at 12,000 × g for 5 min and analyzed by HPLC as described above. For the recycling of the catalyst, the whole cell and supernatant are separated by centrifugation at the end of the batch reaction. Add a new reaction solution to the cell and restart the catalysis process.

**RESULTS AND DISCUSSION**

**Substrate Specificity and Enantioselectivity of Est924**

It is widely known that bHSLs are promiscuous by nature (Pascale and Arnaud, 2012). For instance, esterases EH1 and EstD11 belong to the bHSL family and demonstrate promiscuous substrate specificity (Martínez-Martínez et al., 2018; Miguel-Ruano et al., 2021). Organic solvents and the alcohol moiety of esters often affect the enantioselectivity of esterases. Est25, EstD11, and Est924 have relatively low (R)-enantioselectivity to ethyl 2-arylpropionates, for this reason are the suitable template for the engineering of an (S)-enantioselective biocatalyst. Methanol, DMSO, and Triton-X100 are good cosolvents for Est924. However, the addition of methanol produces transesterified products that are difficult to be separated from the reaction mixture (data not shown). The foam formed by Triton-X100 is not conducive to industrial applications. Therefore, ethyl 2-arylpropionates and DMSO were selected as the substrates and cosolvent, respectively. The substrate specificity of Est924 was determined at 30°C in pH 8.0 buffer by using racemic ethyl (R, S)-2-arylpropionates as substrates. As shown in Figure 1 and Table 1, Est924 could hydrolyze all tested substrates, indicating that it had a wide substrate scope. In the pharmaceutical industry, there is an increasing demand for esterase-mediated chiral resolution of racemic 2-arylpropionate esters due to the high activity and low side effects of the (S)-enantiomers. The enantioselectivity of Est924 was subsequently determined using (R) and (S)-naproxen ethyl ester as substrates. Similar to Est25 (Kim et al., 2006) and SsoEST1 (Sehgal and Kelly, 2003), wild-type Est924 had activity toward (R)-naproxen ethyl ester and (S)-naproxen ethyl ester, with a slight preference for (R) -enantiomer (10%ee(R)).

**Structural Analysis of bHSL Family Esterases**

Enzymes with broad-spectrum substrate applicability generally owe this to their larger active pocket, similar to bHSL family esterases (Martínez-Martínez et al., 2018). According to the classification of the Esther database (Lenfant et al., 2013), 111 esterase structures of the bHSL family were resolved. We superimposed the crystal structures of Est25 (PDB: 4J7A) and 41 wild-type enzymes of bHSL family (Supplementary Figure 2). Three-quarters of bHSL family esterases had highly similar crystal structures, although the lowest sequence similarity observed between 5IQ3 and 3D7R was only 11.7%
The Enzyme Activity and Enantioselectivity of Esterases

The enzyme activity and enantioselectivity of esterases were measured using \((R, S)\)-naproxen ethyl ester as substrate by HPLC. Firstly, A203 was mutated to tryptophan according to the structural analysis and results obtained by Kim et al. (2017). As predicted, variation of residue A203 significantly impacts enantioselectivity, and the variant M1 (A203W) turned to \((S)\)-selective, producing \((S)\)-naproxen with 65.6\%ee\(_p\). The variant Est924\(_{F72G}\) also reversed the stereoselectivity and hydrolyzed \((S)\)-naproxen ethyl ester with moderate selectivity, yielding \((S)\)-naproxen with 56.3\%ee\(_p\). However,
M1, G208F, M2, and M3 with (R)-(mM/mg/min) for biocatalytic reactions using wild-type Est924, reversed and increased, we investigated the specific activity 95.7% high as M2, and variant M3 (I202F/A203W/G208F) with mutation at I202 still retained the (S)-naproxen with (A203W and G208F was highly (S)-selective, producing (S)-naproxen ethyl ester as substrates. Est924 showed the lowest enantioselectivity among esterases (Table 3, list 1), which mainly caused similar hydrolysis activity as compared to the enantiomers. M1 retained 90% activity of Est924 on (S)-naproxen ethyl ester, and 15% activity against (R)-enantiomer (Table 3, list 2). Therefore, the enantioselectivity of M1 was reversed and (S)-naproxen was formed as the main product. M3 showed the highest enantioselectivity among the esterases examined. It was almost inactivated in its ability to process (R)-naproxen ethyl ester, but still retained 80% of the activity of Est924 when (S)-naproxen ethyl ester was used as a substrate (Table 3, list 5). Subsequently, the structure of the optimal variant M3 was simulated and docked with (R) and (S)-naproxen ethyl ester, respectively. As shown in Figure 4A, (S)-naproxen ethyl ester fitted well with the active pocket but failed to obtain an effective docking of (R)-enantiomer with M3. Next, we docked (R) and (S)-naproxen ethyl ester into the structure of Est924, as shown in Figure 4B. Both the (R) and (S)-enantiomer pointed their naphthalene rings to the acyl binding site region. The distance between the protonated Nε atom of the active histidine residue and the ester oxygen atom (dNE−O) was usually taken as a geometrical probe to indicate enantioselectivity (Henke et al., 2003). The dNE−O of (R)-naproxen ethyl ester (3.3Å) was 0.3Å shorter than (S)-naproxen ethyl ester (3.6Å), which corresponded to the slight (R)-stereopreference of Est924. We superimposed the modeled structure of M3 and Est924, and found that the α-methyl of (R)-naproxen ethyl ester was blocked by the steric hindrance of residue W203, 

the variant Est924I212F with a larger hydrophobic side chain did not affect the enantioselectivity. These results indicated that both positions (204 and 208) were effective in improving the selectivity of Est924. Variant M2 with A203W and G208F was highly (S)-selective, producing (S)-naproxen with 95%ee. Finally, the variant with a mutation at I202 still retained the (S)-enantioselectivity as high as M2, and variant M3 (I202F/A203W/G208F) with 95.7%ee was obtained.

To clarify why the enantioselectivity of the variants was reversed and increased, we investigated the specific activity (mM/mg/min) for biocatalytic reactions using wild-type Est924, M1, G208F, M2, and M3 with (R)-naproxen ethyl ester and (S)-naproxen ethyl ester as substrates. Est924 showed the lowest enantioselectivity among esterases (Table 3, list 1), which mainly caused similar hydrolysis activity as compared to the enantiomers. M1 retained 90% activity of Est924 on (S)-naproxen ethyl ester, and 15% activity against (R)-enantiomer (Table 3, list 2). Therefore, the enantioselectivity of M1 was reversed and (S)-naproxen was formed as the main product. M3 showed the highest enantioselectivity among the esterases examined. It was almost inactivated in its ability to process (R)-naproxen ethyl ester, but still retained 80% of the activity of Est924 when (S)-naproxen ethyl ester was used as a substrate (Table 3, list 5). Subsequently, the structure of the optimal variant M3 was simulated and docked with (R) and (S)-naproxen ethyl ester, respectively. As shown in Figure 4A, (S)-naproxen ethyl ester fitted well with the active pocket but failed to obtain an effective docking of (R)-enantiomer with M3. Next, we docked (R) and (S)-naproxen ethyl ester into the structure of Est924, as shown in Figure 4B. Both the (R) and (S)-enantiomer pointed their naphthalene rings to the acyl binding site region. The distance between the protonated Nε atom of the active histidine residue and the ester oxygen atom (dNE−O) was usually taken as a geometrical probe to indicate enantioselectivity (Henke et al., 2003). The dNE−O of (R)-naproxen ethyl ester (3.3Å) was 0.3Å shorter than (S)-naproxen ethyl ester (3.6Å), which corresponded to the slight (R)-stereopreference of Est924. We superimposed the modeled structure of M3 and Est924, and found that the α-methyl of (R)-naproxen ethyl ester was blocked by the steric hindrance of residue W203,

### Table 3 Comparison of enzyme activity and enantioselectivity of Est924 and its mutants.

| Entry | Esterase | Specific activity (mM/mg/min) [a] | (R)-Naproxen | (S)-Naproxen | Conversion/% [b] | E value[c] |
|-------|----------|----------------------------------|--------------|--------------|----------------|----------|
| 1     | Est924   | 3.5                              | 3.3          |              | 45             | 1.3 [R]  |
| 2     | A203W    | 0.5                              | 3.1          |              | 38             | 7 [S]   |
| 3     | G208F    | 0.8                              | 3.2          |              | 33             | 4.6 [S] |
| 4     | M2       | 0.04                             | 2.6          |              | 31             | 59 [S]  |
| 5     | M3       | 0.02                             | 2.5          |              | 30             | 68 [S]  |

[a] Reactions were carried out under the optimal conditions.

[b] Using racemic naproxen ethyl ester as substrate.

[c] E values were calculated refers to the method of Ngo et al. (2019).
which prevented it from being effectively bound. In addition, the molecular docking results also suggested that (S)-naproxen ethyl ester was not affected by the newly generated steric hindrance from large side chain amino acids in M3. However, mutations reduced the activity pockets of enzymes which were not conducive to the entry of substrates, so their activities on (S)-naproxen ethyl ester were reduced. This suggested that the enantioselectivity reversal of the enzyme was due to the steric hindrance effect that made it difficult for the enzyme to bind (R)-enantiomer, which was consistent with the results of specific activity tests.

**Resolution of Racemic Ibuprofen Ethyl Ester and Ketoprofen Ethyl Ester With M3**

In addition to (S)-naproxen, (S)-ibuprofen and (S)-ketoprofen are also widely used. A variety of esterases have been used

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**FIGURE 4 |** Docking models of (R) and (S)-naproxen ethyl ester to Est924 and mutant M3. (A) Detailed interactions of the docking (S)-naproxen ethyl ester (sand stick) and residues in the active site of M3 (gray stick representation). (B) Docking of (R)-naproxen ethyl ester (green stick) and (S)-naproxen ethyl ester (sand stick) into the active site of the Est924 model. Catalytic residues (S152 and H279) were shown as white sticks, the residues (G80, H90, and D151) forming the oxyanion hole was shown as cyan sticks. The residues around Cα of naproxen ethyl esters were shown as yellow sticks. The distance between the protonated Nε atom of the active histidine residue and the ester oxygen atom (dNε−O) of (R)- and (S)-naproxen ethyl ester were shown as green dashed lines.

**FIGURE 5 |** Repeated use of cells. The reaction mixture containing 50 mM potassium phosphate buffer (pH 8.0), 10 mM naproxen ethyl ester (A) or ketoprofen ethyl ester (B), and 5% DMSO to form cell suspension (OD600 nm = 3) with a total volume of 10 mL (in a 50 mL shake flask). The bioconversion reactions were performed at 30°C and 200 rpm for 1 h (ketoprofen ethyl ester) and 1.5 h (naproxen ethyl ester) in each cycle respectively. Royal blue bars, conversion of rac-ketoprofen ethyl ester and naproxen ethyl ester; Salmon point-plot, E values of (S)-ketoprofen and (S)-naproxen.
to produce them through the resolution of corresponding racemic esters (Kim et al., 2002; Choi et al., 2003; Sathishkumar et al., 2010; López et al., 2014; Yoon et al., 2014; Memarpour-Yazdi et al., 2018). Est924 could effectively hydrolyze both racemic ibuprofen ethyl ester and ketoprofen ethyl ester (Table 1). The optimal variant (M3) was tested for enantioselective resolution of these racemic esters. The results showed that M3 preferred to hydrolyze their (S)-conformation esters, generating (S)-ibuprofen (74.5%ee) and (S)-ketoprofen (95%ee), respectively. These results showed that we successfully generated Est924 variants with inverted and highly increased enantioselectivity toward ethyl (S)-2-arylpromonates and provide Est924 mutants with potential application in industrial biocatalysts. On the other hand, the low enantioselectivity of M3 to ibuprofen ethyl ester also suggested that the stereoselectivity of the enzyme to ethyl 2-arylpromonates was also regulated by other residues in the active pocket, especially the acyl binding site.

**Synthesis of (S)-Ketoprofen and (S)-Naproxen by Whole-Cell Catalysis**

Compared with free enzyme and immobilized enzyme, whole-cell catalysts avoided the separation and purification of enzymes, which might be the better method for reducing the cost of the process. Ketoprofen ethyl ester and naproxen ethyl ester were selected as substrates due to the high enantioselectivity of M3. Control cells harboring the empty vector (in the absence of M3) were verified to catalyze the conversion of ketoprofen ethyl ester and naproxen ethyl ester was less than 1% within 24 h, which indicated that the whole-cell catalyst did not have undesired reactions. Besides, although the cell envelope of the whole-cell catalysts helps stabilize enzymes, it reduces the mass transfer efficiency of substrates and products. Considering the activity and recovery of the whole-cell catalyst, freeze-thaw treatment of *E. coli* cells was a common method (Schwaiger et al., 2021). Using cell lysate as a reference, the cells achieved about 81% of the relative activity and still maintained high (S)-enantioselectivity toward ketoprofen ethyl ester (95%ee) and naproxen ethyl ester (96%ee) after a single freeze-thaw cycle. The recyclability of biocatalyst was an important factor in the industrial application of the whole-cell biocatalytic process. As shown in Figure 5, the cells could be reused for at least five cycles without sacrificing the activity and enantioselectivity.

**CONCLUSION**

In this study, we carefully analyzed the structure of 41 wild-type esterases of the bHSL family. Their crystal structures are highly similar, although with only low to moderate sequence similarity. Several residues that may reverse and enhance the enantioselectivity of the enzyme toward (S)-2-aryl propionate esters were found through docking (R) and (S)-naproxen ethyl ester into the crystal structure of Est25. Est924, an esterase of the bHSL family, was set as the starting enzyme, because it could hydrolyze a variety of ethyl 2-arylpromonates with light (R)-enantioselectivity. By protein engineering, the key position A2 was substituted by tryptophan, and the substrate binding pockets were reshaped due to its large and hydrophobic side chain; this switched its stereoselectivity to (S)-2-aryl propionate esters. Another key residue at position B1 was mutated to phenylalanine, which further improved the stereoselectivity of the enzyme. Mutant M3 has A2 and B1 di-substitution, could resolve racemic naproxen ethyl ester, ketoprofen ethyl ester and ibuprofen ethyl ester with moderate to high (S)-enantioselectivity. In addition, an easy-to-perform whole-cell catalytic approach was successfully developed and used in the synthesis of (S)-ketoprofen and (S)-naproxen.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

**AUTHOR CONTRIBUTIONS**

XL analyzed the structures, screened the key residues, and performed HPLC analysis. MZ constructed site mutations, screened clones, and tested activity and selectivity. XF written and revised the manuscript. YF conceived the study and supervised the experiments. All authors have read and approved the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2021.697677/full#supplementary-material

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**REFERENCES**

Adams, J. P., Brown, M. J. R., Diaz-Rodriguez, A., Lloyd, R. C., and Roiban, G. D. (2019). Biocatalysis: a pharma perspective. *Adv. Synth. Catal.* 361:2421. doi: 10.1002/adsc.201900424

Choi, G. S., Kim, J. Y., Kim, J. H., Ryu, Y. W., and Kim, G. J. (2003). Construction and characterization of a recombinant esterase with high activity and enantioselectivity to (S)-ketoprofen ethyl ester. *Protein Expr. Purif.* 29, 85–93. doi: 10.1016/S1046-5928(03)00099-3

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Liu et al.
Liu, Y., Liu, X., Zhao, M., and Fan, X. (2020). The enzymatic properties of esterase Lin, B., and Tao, Y. (2017). Whole-cell biocatalysts by design. Laskowski, R. A., Rullmannn, J. A., Macarthur, M. W., Kaptein, R., and Thornton, J. M. (1996). AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. J. Biomol. NMR 8, 477–486. doi:10.1007/bf00228148 Lenfant, N., Hotelier, T., Velluet, E., Bourne, Y., Marchot, P., and Chatonnet, A. (2013). ESTHER, the database of the α/β-hydrolase fold superfAMILY of proteins: tools to explore diversity of functions. Nucleic Acids Res. 41, D423–D429. doi:10.1093/nar/gks1154 Lin, B., and Tao, Y. (2017). Whole-cell biocatalysts by design. Microb. Cell Factories 16:106. doi:10.1186/s12934-017-0724-7 Liu, Y., Liu, X., Zhao, M., and Fan, X. (2020). The enzymatic properties of esterase from metagenomic sources and the degradation of phthalates. Acta Sci. Nat. Univ. Sunyatseni. 59, 41–50. López, G., Chow, J., Bongen, P., Lausinger, B., Pietruszka, J., Streit, W. R., et al. (2011). A novel thermoalkalostable esterase from Acidicilus sp. strain USBA-GBX-499 with enantioselectivity isolated from an acidic hot springs of Colombian Andes. Appl. Microbiol. Biotechnol. 98, 8603–8616. doi:10.1007/s00253-014-5775-7 Lough, W. J., Drayer, D., Brittain, H., Iozwiak, K., Wainer, L., Cox, G., et al. (2012). Drug Stereochemistry, Analytical Methods and Drug Pharmacology, London: CRC Press.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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