Ultrasound promoted enantioselective transesterification of 3-hydroxy-3-(2-thienyl) propanenitrile catalyzed by lipase

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ABSTRACT

(S)-3-hydroxy-3-(2-thienyl) propanenitrile, which is the key chiral building block for the synthesis of (S)-duloxetine, was successfully prepared via enantioselective transesterification catalyzed by lipase under ultrasound irradiation. Compared with conventional shaking, the enzyme activity and enantioselectivity were dramatically enhanced under ultrasound irradiation. Under optimum reaction conditions (solvent: n-hexane, ultrasound power: 150 W, a_w: 0.33, temperature: 40°C), Pseudomonas sp. lipase exhibited an excellent catalytic performance (enzyme activity: 81.5 μmol g⁻¹ min⁻¹, E-value: 65.4). The reaction achieved its equilibrium in approximately 7 h with a conversion of 53.9% and high enantiopurity (99% ee) of (S)-3-hydroxy-3-(2-thienyl) propanenitrile could be obtained.

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Introduction

Duloxetine is regarded as a useful drug for the treatment of psychiatric and metabolic disorders (1, 2). Its biological activity resides mainly in its (S)-enantiomer (3), which can be prepared from (S)-3-hydroxy-3-(2-thienyl) propanenitrile (4). However, it is difficult to prepare the chiral intermediate with high enantiopurity via chemical synthesis (5–7). Moreover, the chemical synthesis needs an expensive chiral catalyst, which is inefficient and toxic. It’s well known that biocatalysis is one of the attractive technologies for the synthesis of chiral molecules due to its exquisite regioselectivity and stereoselectivity under mild conditions (8). Träff et al. have described a dynamic kinetic resolution of 3-hydroxy-3-(2-thienyl) propanenitrile using Candida antarctica lipase B and ruthenium catalyst, which can afford (S)-3-hydroxy-3-(2-thienyl) propanenitrile (98% ee) in high yield (9). Ahmed Kamal and his co-workers have reported another efficient enzymatic synthesis for preparing the optically pure (S)-enantiomer of 3-hydroxy-3-(2-thienyl) propanenitrile catalyzed by lipase PS-D (Pseudomonas cepacia lipase immobilized on diatomite) in 14 h (10). Nevertheless, the unsatisfied reaction rate or enantioselectivity in the reported enzymatic resolutions in nonaqueous media remains a pressing concern.

As an environmentally benign method, ultrasound irradiation has been successfully introduced into enzymatic reactions to improve the enzyme performance and shorten the reaction time in the past few years (11). For instance, ultrasound irradiation has been successfully employed in the enzymatic processes such as synthesis of sugar esters (12), synthesis of saturated aliphatic esters (13) and the alcoholysis for the production of biodiesel (14, 15). Our previous reports have also demonstrated that ultrasound can be applied to improve the catalytic efficiency of enzyme for the resolution of chiral compounds (16–18).

In order to obtain (S)-3-hydroxy-3-(2-thienyl) propanenitrile with high enantiopurity in a short time, ultrasound was adopted in this study to improve the enzyme performance and the reaction conditions have also been optimized (Scheme 1).

Results and discussion

Generally, the catalytic performance of lipase depended mainly on its type and origin (19). In this work, different
lipases have been selected to investigate their performance under ultrasound. As shown in Table 1, the control experiment did not show any conversion and *Pseudomonas* sp. lipase (PSL) exhibited the highest enantioselectivity and enzyme activity under ultrasound irradiation among the selected lipases.

Different ultrasound powers (100, 125, 150, 175, 200, 225, 250 W) were selected to examine its effect on the enantioselective transesterification. The results in Figure 1 demonstrate that a suitable ultrasound power was necessary for this study. The highest enantioselectivity was obtained at 150 W. And the enzyme activity increased when the ultrasound power was enhanced from 100 to 200 W and decreased under higher power. Ultrasound can decrease the enzyme aggregation and mass transfer limitation. Furthermore, the cavitation caused by ultrasound can increase the chance of the substrate’s access to the active site of lipase. In addition to promoting the activity, ultrasound also affected the enantioselectivity of lipase. When the power of ultrasound was increased, the flexibility of the enzyme conformation might be improved, which could lead to an enhancement of the enantioselectivity. But high ultrasound power might disrupt the hydrogen bonding or *van der Waals* interactions of protein and destroy the active conformation of enzyme, and then decrease the activity and enantioselectivity of lipase. Furthermore, the shear force which occurs in the reaction media under ultrasound may also play a significant role in enzyme inactivation. Consequently we selected 150 W as the optimal ultrasound power in the following experiments.

In this study, the effects of the solvent with different log *P* (logarithm of the partition coefficient of a given solvent between *n*-octanol and water) were investigated. As shown in Table 2, the hydrophobicity of solvent could influence enzyme performance obviously. The activity and enantioselectivity increased with the increasing of log *P*. Organic solvents with low log *P* can strip the essentially bound water molecules from the enzyme surface due to their high affinity to water, and then lead to the inactivation of the enzyme (20). Furthermore, this kind of solvents could also change the active conformation of PSL and then decrease its enantioselectivity. Water can influence the activity and enantioselectivity, as well

![Scheme 1. Ultrasound promoted transesterification of 3-hydroxy-3-(2-thienyl) propanenitrile catalyzed by lipase.](image)

**Table 1. Effect of enzyme type on the enzymatic transesterification of 3-hydroxy-3-(2-thienyl) propanenitrile.**

| Enzyme                     | Conversion (%) | Enzyme activity (μmol g⁻¹ min⁻¹) | ee, (%) | Enantioselectivity (E-value) |
|----------------------------|----------------|----------------------------------|---------|-----------------------------|
| *Candida antarctica* lipase B (CalB) | 16.5 ± 1.1     | 69.1 ± 2.3                       | 19.0 ± 1.7 | 58.3 ± 3.2 |
| *Pseudomonas* sp. Lipase (PSL) | 23.2 ± 0.9     | 81.5 ± 1.3                       | 29.0 ± 1.3 | 65.4 ± 1.9 |
| *Bacillus subtilis* lipase (BSL2) | 19.2 ± 1.5     | 39.4 ± 2.8                       | 21.8 ± 2.2 | 28.6 ± 4.2 |
| *C. rugosa* lipase (CRL)       | 25.6 ± 0.7     | 66.8 ± 1.3                       | 30.4 ± 1.8 | 21.9 ± 2.0 |
| Porcine pancreas lipase (PPL)  | 15.4 ± 0.8     | 28.6 ± 3.1                       | 16.9 ± 2.4 | 30.8 ± 3.5 |
| Lipase from *Candida* sp.      | 18.2 ± 1.4     | 44.7 ± 2.4                       | 19.8 ± 0.9 | 20.7 ± 2.7 |
| Control*                     | ND             | ND                               | ND       | ND                          |

Notes: Reaction condition: the reactions were carried out in *n*-hexane (10 mL) with 3-hydroxy-3-(2-thienyl) propanenitrile (1 mmol), vinyl acetate (3 mmol), enzyme (20 mg) and *aw* (0.33) under ultrasound (150 W, 40°C). ND, not detected.

*Control: without lipase.*

![Figure 1. Effect of ultrasound power on the enzymatic transesterification of 3-hydroxy-3-(2-thienyl) propanenitrile reaction condition: the reactions were carried out in *n*-hexane (10 mL) with 3-hydroxy-3-(2-thienyl) propanenitrile (1 mmol), vinyl acetate (3 mmol), PSL (20 mg) and *aw* (0.33) under ultrasound (40°C).](image)
as the stability of enzymes, and it is also a competitive nucleophile in a lipase-catalyzed transesterification (21). Here, the effect of water activity (a_w) on the enzyme performance in the transesterification under ultrasound was investigated and the results are shown in Figure 2. Maximum enzyme activity was observed at a_w = 0.53 and the highest E-value was observed at a_w = 0.33. Water activity could influence the conformation rigidity and disturb the ‘induced-fit’ process of lipase, which might change the enzyme performance (22). Furthermore, high a_w would lead to the aggregation of the enzyme particle that may, in turn, limit the access of the substrate into the enzyme active site. Since E-value (65.4) was found to be the highest at a_w = 0.33 while maintaining a higher enzyme activity (81.5 μmol g⁻¹ min⁻¹), 0.33 was selected as the optimal water activity for this reaction.

The effects of temperature were examined in a range of 20–60°C under ultrasound. The result in Figure 3 shows that the enantioselectivity exhibited a bell-shaped curve with the change in temperature and PSL exhibited the highest enzyme enantioselectivity at 40°C. The enzyme activity increased as temperature increased from 20°C to 40°C, and then decreased at higher temperatures. When the reaction temperature was elevated, the collision between enzyme and substrate molecules was increased which could help to form enzyme-substrate complexes and then enhance the enzyme activity. The activity significantly decreased at higher temperatures, which might be due to the denaturation of the lipase, especially under ultrasound irradiation.

The effect of mole ratio (vinyl acetate/3-hydroxy-3-(2-thienyl) propanenitrile) was investigated when the amount of the enzyme and the concentration of 3-hydroxy-3-(2-thienyl) propanenitrile were kept constant (Figure 4). It was shown experimentally that the enzyme activity was gradually improved with increasing the substrate ratio from 1 : 1 to 3 : 1. The enzyme activity leveled off after this point (3 : 1). The adding of excess vinyl ester during reaction could inhibit the hydrolysis of esters and thus raises the reaction equilibrium as well as increasing the theoretical maximum product yield. On the other hand, the enantioselectivity was not affected by changing the substrate ratio. Besides, the effect of the amount of PSL was also investigated when the substrate ratio was fixed at 3 : 1 (data not shown here). It was found that 2 mg mL⁻¹ PSL was sufficient for the resolution in

| Solvent       | Log p | Conversion (%) | Enzyme activity (μmol g⁻¹ min⁻¹) | ee (%) | E-value |
|---------------|-------|---------------|----------------------------------|--------|---------|
| 1,4-Dioxane   | −1.1  | 20.8 ± 1.8    | 10.8 ± 2.9                       | 24.6 ± 1.3 | 37.8 ± 3.8 |
| Acetonitrile  | −0.33 | 15.4 ± 2.1    | 19.7 ± 2.4                       | 17.3 ± 1.1 | 43.4 ± 3.1 |
| Tetrahydrofuran | 0.49 | 17.5 ± 1.6    | 39.2 ± 2.5                       | 20.2 ± 1.9 | 48.7 ± 3.8 |
| Cyclohexane   | 1.2   | 26.2 ± 1.4    | 60.3 ± 1.2                       | 33.7 ± 0.6 | 51.8 ± 1.8 |
| Toluene       | 2.5   | 16.3 ± 1.3    | 67.9 ± 1.4                       | 18.8 ± 0.8 | 64.9 ± 2.0 |
| n-Hexane      | 3.5   | 23.2 ± 0.9    | 81.5 ± 1.3                       | 29.0 ± 1.3 | 65.4 ± 1.9 |

Note: Reaction condition: the reactions were carried out in organic solvent (10 mL) with 3-hydroxy-3-(2-thienyl) propanenitrile (1 mmol), vinyl acetate (3 mmol), PSL (20 mg) and a_w (0.33) under ultrasound (150 W, 40°C).

Figure 2. Effect of water activity on the enzymatic transesterification of 3-hydroxy-3-(2-thienyl) propanenitrile reaction condition: the reactions were carried out in n-hexane (10 mL) with 3-hydroxy-3-(2-thienyl) propanenitrile (1 mmol), vinyl acetate (3 mmol) and PSL (20 mg) under ultrasound (150 W, 40°C).

Figure 3. Effects of temperature on the enzymatic transesterification of 3-hydroxy-3-(2-thienyl) propanenitrile under ultrasound reaction condition: the reactions were carried out in n-hexane (10 mL) with 3-hydroxy-3-(2-thienyl) propanenitrile (1 mmol), vinyl acetate (3 mmol), PSL (20 mg) and a_w (0.33) under ultrasound (150 W).
this study. After 2 mg mL\(^{-1}\), the reaction rate was not increasing obviously.

The substrate concentration may affect the enzyme activity \((23)\). As shown in Figure 5, when the amount of the enzyme and the substrate ratio were kept constant, the enzyme activity was increased with increasing the concentration of 3-hydroxy-3-(2-thienyl) propanenitrile \((0.01 \text{–} 0.1 \text{ mmol mL}\(^{-1}\))\). But higher substrate concentration \((>0.1 \text{ mmol mL}\(^{-1}\))\) cannot enhance the enzyme activity further. The results also suggested that the enantioselectivity of lipase was not influenced by the substrate concentration apparently.

The enzymatic resolution under ultrasound irradiation and conventional heating was compared. As shown in Table 3, the ultrasound was found to enhance both the enzyme activity and enantioselectivity, compared with the results obtained from conventional heating. Ultrasound can accelerate the reaction by reducing the mass transfer limitations. Furthermore, it could change the enzyme conformation by perturbing the weak interactions, which can affect the enantioselectivity. Compared with the reported literature \((10)\), this ultrasound-promoted enzymatic resolution is more efficient and the enantioselectivity is also satisfactory in this work.

Under the optimum conditions, we scaled up the enzymatic process 25-fold \([n\text{-hexane (250 mL), 3-hydroxy-3-(2-thienyl) propanenitrile (25 mmol), vinyl acetate (75 mmol), PSL (500 mg), ultrasound power (150 W), water activity (0.33), temperature (40°C)$\] with a conversion of 53.9% and 99%\text{ee} of the unreacted \((S)-3\text{-hydroxy-3-(2-thienyl) propanenitrile}$ (Figure 6). As for the conventional heating, the unreacted substrate with high enantiopurity (99%\text{ee}) could be obtained when the reaction time was prolonged to 20 h (conversion: 54.8%). These results obviously demonstrated that ultrasound could improve the enzyme performance and shorten the reaction time.

### Experimental

#### Materials

*Candida* sp. lipase was obtained from Beijing CTA New Century Biotechnology Co. Ltd (Beijing, China). *Bacillus subtilis* lipase (BSL2) was over-expressed from *Bacillus subtilis* strain IFFI10210 in our laboratory according to the method we have reported \((24)\). These enzymes were used after lyophilization for enzymatic reaction.

### Table 3. Comparison of the enzymatic resolution heated by ultrasound irradiation and conventional heating.

| Condition          | Conversion (%) | Enzyme activity (μmol g\(^{-1}\) min\(^{-1}\)) | ee, (%) | Enantioselectivity (E-value) |
|--------------------|----------------|---------------------------------------------|---------|-----------------------------|
| Conventional heating* | 18.7 ± 1.4  | 28.7 ± 1.8          | 21.9 ± 1.1  | 51.2 ± 2.1  |
| Ultrasound irradiation\(b\) | 23.2 ± 0.9  | 81.5 ± 1.3          | 29.0 ± 1.3  | 65.4 ± 1.9  |

\(a\)Reaction condition: the reactions were carried out in \(n\text{-hexane (10 mL), 3-hydroxy-3-(2-thienyl) propanenitrile (1 mmol), vinyl acetate (3 mmol), PSL (20 mg) and }\(a_w\) (0.33) at 40°C.

\(b\)Reaction condition: the reactions were carried out in \(n\text{-hexane (10 mL), 3-hydroxy-3-(2-thienyl) propanenitrile (1 mmol), vinyl acetate (3 mmol), PSL (20 mg) and }\(a_w\) (0.33) under ultrasound (150 W, 40°C).
without further purification. 4-Crotonaldehyde was purchased from Sigma. Other reagents were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Commercially available reagents and solvents were used without further purification. The ultrasound bath, KQ-250DE (Kunshan Ultrasound Co., Ltd., China), was basically a rectangular container. The temperature was controlled by circulating water.

Water activity setting
All the reaction mixture components and the enzyme powder were pre-equilibrated to the desired water activity (a_w) with saturated salt solutions at 25°C in the separate containers: LiBr (a_w = 0.06), LiCl (a_w = 0.11), KCH_3COO (a_w = 0.24), MgCl_2 (a_w = 0.33), Mg(NO_3)_2 (a_w = 0.53), NaCl (a_w = 0.75), K_2SO_4 (a_w = 0.97). The equilibration was performed overnight.

Enzymatic transesterification of 3-hydroxy-3-(2-thienyl) propanenitrile
The reaction was performed by using (R/S)-3-hydroxy-3-(2-thienyl) propanenitrile (1 mmol), vinyl acetate (3 mmol), n-hexane (10 mL) and PSL (20 mg) with stirring or in an ultrasound bath (150 W) at 40°C. To determine the conversion of 3-hydroxy-3-(2-thienyl) propanenitrile, the organic samples were withdrawn from the reaction mixture, and analyzed by high-performance liquid chromatography (HPLC). The enzyme activity (μmol g⁻¹ min⁻¹) was defined as the amount (in micromoles) of the produced ester per minute per gram of protein content.

Determination of enantiomeric excess and enantioselectivity
The samples were withdrawn from the vials and analyzed directly by chiral HPLC (chiral OJ-H column, Diacel) employing hexane-isopropanol (85:15) as mobile phase at 0.75 mL min⁻¹ and monitored by UV (254 nm). The degree of conversion (C) was calculated from the reduction of 3-hydroxy-3-(2-thienyl) propanenitrile. The enantiomeric excess (ee) was determined by calculating the peak areas of the two enantiomers and the enantiomeric ratio (E-value) was determined from C and (ee) by using equation (1) (25):

\[
\text{Enantiomeric excesses, } ee_i (\%) = \frac{[S - R]}{[S + R]} \times 100, \\
\text{Enantioselectivity, } E = \frac{\ln [(1 - C)(1 - ee)]}{\ln [(1 - C)(1 + ee)]}.
\]

Conclusion
In summary, we used ultrasound irradiation to improve the enzyme activity and enantioselectivity of PSL in the enzymatic transesterification of 3-hydroxy-3-(2-thienyl) propanenitrile. Under optimum conditions [n-hexane (10 mL), 3-hydroxy-3-(2-thienyl) propanenitrile (1 mmol), vinyl acetate (3 mmol), PSL (20 mg), ultrasound power (150 W), water activity (0.33), temperature (40°C)], PSL exhibited an excellent catalytic performance (enzyme activity: 81.5 μmol g⁻¹ min⁻¹, E-value: 65.4). Compared with conventional shaking, the enzyme activity and enantioselectivity were increased about 2.84-fold and 1.28-fold, respectively.

Disclosure statement
No potential conflict of interest was reported by the authors.

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