Received: 13 July 2017 | Revised: 28 August 2017 | Accepted: 3 September 2017

DOI: 10.1002/chir.22771

REGULAR ARTICLE

WILEY

Exploration of the expeditious potential of *Pseudomonas fluorescens* lipase in the kinetic resolution of racemic intermediates and its validation through molecular docking

Surbhi Soni[2] | Bharat P. Dwivedee[1] | Vishnu K. Sharma[3] | Gopal Patel[1] | Uttam C. Banerjee[1]

[1] Department of Pharmaceutical Technology (Biotechnology), National Institute of Pharmaceutical Education and Research, Punjab, India

[2] Department of Biotechnology, National Institute of Pharmaceutical Education and Research, Punjab, India

[3] Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research, Punjab, India

Correspondence
U.C. Banerjee, Department of Pharmaceutical Technology (Biotechnology), National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S.Nagar-160062, Punjab, India.
Email: ucbanerjee@niper.ac.in

Abstract

A profoundly time-efficient chemoenzymatic method for the synthesis of (*S*)-3-(4-chlorophenoxy)propan-1,2-diol and (*S*)-1-chloro-3-(2,5-dichlorophenoxy)propan-2-ol, two important pharmaceutical intermediates, was successfully developed using *Pseudomonas fluorescens* lipase (PFL). Kinetic resolution was successfully achieved using vinyl acetate as acylating agent, toluene/hexane as solvent, and reaction temperature of 30°C giving high enantioselectivity and conversion. Under optimized condition, PFL demonstrated 50.2% conversion, enantiomeric excess of 95.0%, enantioselectivity (E = 153) in an optimum time of 1 hour and 50.3% conversion, enantiomeric excess of 95.2%, enantioselectivity (E = 161) in an optimum time of 3 hours, for the two racemic alcohols, respectively. Docking of the *R*- and *S*-enantiomers of the intermediates demonstrated stronger H-bond interaction between the hydroxyl group of the *R*-enantiomer and the key binding residues of the catalytic site of the lipase, while the *S*-enantiomer demonstrated lesser interaction. Thus, docking study complemented the experimental outcome that PFL preferentially acylated the *R* form of the intermediates. The present study demonstrates a cost-effective and expeditious biocatalytic process that can be applied in the enantiopure synthesis of pharmaceutical intermediates and drugs.

KEYWORDS

biocatalysis, chemoenzymatic synthesis, enantiomeric excess, enantioselectivity, β-aryloxyalcohols

1 | INTRODUCTION

Biocatalysis has become a key strategy for the synthesis of chiral drugs. The foremost reason for the wide application of biocatalysis in synthetic reactions is the high degree of selectivity along with ecological benefits.[25] Lipases are the most common enzymes applied in biocatalysis. Lipases have achieved a distinguished position owing to their versatile nature and easy availability. Over the years, several lipase-catalyzed processes for the synthesis of enantiopure drugs and drug intermediates have emerged.[15] The stability of lipases in organic solvents has further widened the scope of their application, making lipase an excellent biocatalyst for the synthesis of single stereoisomers of racemic drugs.[16] Among the lipases applied in biotransformations, the lipase from *Pseudomonas fluorescens* (PFL) is an interesting candidate. PFL displays specialities in the kinetic resolution[13] of racemic alcohols, acids, and esters, as well as in the large-scale synthesis of bulk products like vitamins[4] and biodiesel.[38] PFL has successfully

 © 2017 Wiley Periodicals, Inc.

been applied in the enantiopure synthesis of intermediates of important beta blocker drugs like propanolol[44], atenolol[2], and CNS drug intermediates like 3-hydroxymethyl-1-tetralone tosylates[6] antifungal *N*-substituted benzimidazole derivatives[24] and has also been used at an industrial scale for the synthesis of Carbovir, an antiviral agent.[26] Several additions to the biocatalytic toolbox of PFL have been made by immobilization of PFL on various matrices like nanoscaffolds, polymeric supports, and hydrophobic sol-gels.[5,8]

β-Aryloxyalcohols are key intermediates in the synthesis of many important drugs.[30] It has been well established that the desirable therapeutic activities of β-aryloxyalcohols reside mainly in the (*S*)-enantiomers.[27,42,43] The chemical synthesis of enantiopure β-aryloxyalcohols was performed by the reaction of substituted phenols with chiral epichlorhydrin or glycidol, to give epoxide intermediates[28,46] followed by the ring opening of these epoxides.[3,7] However, the use of chiral reagents makes the process more cost intensive. Here we report an extremely time-efficient, economic, and environmentally benign methodology for the synthesis of two important pharmaceutical intermediates, which depict the promising biocatalytic potential of PFL.

In the present study, two important racemic β-aryloxyalcohols, (*RS*)-1-chloro-3-(2,5-dichlorophenoxy) propan-2-ol [(*RS*)-**4**], and (*RS*)-3-(4-chlorophenoxy)propane-1,2-diol [(*RS*)-**8**] were chemically synthesized and subjected to lipase-catalyzed kinetic resolution. Five commercial lipase preparations were screened. The enantiopure intermediates obtained by lipase-catalyzed kinetic resolution can be used for the enantiopure synthesis of cloranolol (a nonselective β-blocker)[23,40] and chlorphenesin (a muscle relaxant and antifungal).[34] All the reaction parameters were optimized using "one factor at a time" approach. Finally, experimental observations were validated by docking the compounds into the active sites of the lipase.

2 | MATERIALS AND METHODS

2,5-Dicholorophenol, 4-chlorophenol, (*RS*)-epichlorhydrin, (*S*)-epichlorhydrin, (*RS*)-cyclopropylmethanol (glycidol), (*S*)-cyclopropylmethanol (glycidol), *Candida antarctica* lipase (CALA and CALB), *Candida rugosa* lipase, and *P. fluorescens* lipase were purchased from Sigma (St. Louis, Missouri). *Burkholderia cepacia* lipase was purchased from Fluka™. Anhydrous Na_2SO_4, K_2CO_3, TLC plates, and HPLC grade solvents were purchased from Merck (Germany). Silica gel (60-120 mesh) for column chromatography was obtained from SRL (India).

2.1 | Analytical methods

Biocatalytic reactions were incubated in an incubator shaker (Kuhner, Switzerland) at 200 rpm. ^{1}H NMR and ^{13}C NMR were obtained using Bruker DPX 400 (1H 400 MHz) in $CDCl_3$. All the chemical shift values were expressed in δ (ppm) units relative to tetramethylsilane (TMS). HPLC (Shimadzu LC-10AT pump, SPD-10A UV-Vis detector) with Chiralcel® OD-H column (0.46 mm × 250 mm; 5 μm, Daicel Chemical Industries, Japan) and Chiralcel® AD-H column (0.45 mm × 250 mm; 5 μm, Daicel Chemical Industries, Japan) were used to determine the conversion rate enantiomeric excess of the substrates and products.

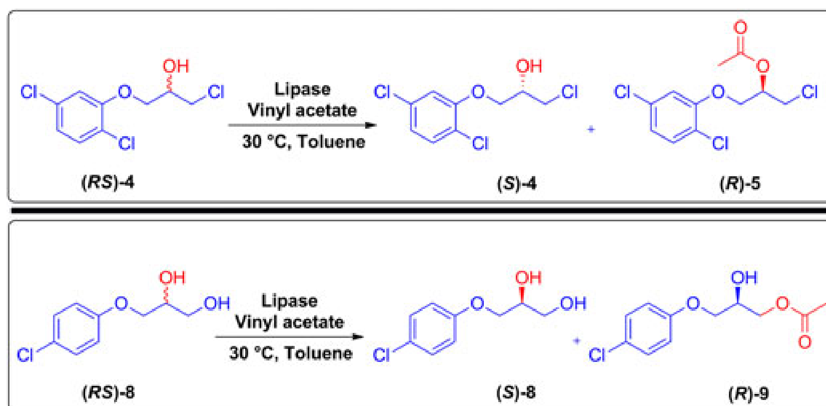
2.2 | Synthesis of (*RS*)-1-chloro-3-(2,5-dichlorophenoxy)propan-2-ol [(*RS*)-4]

The intermediate of cloranolol was prepared via chemical route using the following steps.

The starting racemic epoxide intermediate (*RS*)-**3** was synthesized by dissolving 2,5-dichlorophenol (**1**) (2.5 g, 15 mM, 1 eqv) in anhydrous acetonitrile followed by the addition of K_2CO_3 (4.14 g, 15 mM, 2 eqv) at 80°C. After stirring the reaction mixture for 30 minutes, (*RS*)-epichlorhydrin (*RS*)-**2** (1.8 mL, 15 mM, 1.5 eqv) was added dropwise. The reaction mixture was extracted with ethyl acetate after the completion of reaction in 30 hours, and the filtrate was concentrated under vacuum. Recovered product (*RS*)-**3** was subjected to the epoxide ring-opening reaction using acetyl chloride (1.1 mL, 10 mM, 1.5 eqv) in dichloromethane : water (1:1) mixture. After the completion of reaction in 10 hours, the reaction mixture was extracted with dichloromethane, dried using anhydrous Na_2SO_4, and concentrated on a Rotavapor. The product (*RS*)-**4** was purified by column chromatography using hexane : ethyl acetate (94:6) as an eluent on silica gel. (*RS*)-**4** was used as the substrate for lipase-catalyzed kinetic resolution (Scheme 1). Using similar method the enantiopure intermediate of cloranolol, (*R*)-**4** was synthesized.

(*RS*)-**3**, light yellow liquid (96% yield), ^{1}H NMR (400 MHz; $CdCl_3$) δ (ppm): 7.28 (dd, 1H, Ar–H), 7.25 (dd, 1H, Ar–H), 6.93 (m, 1H, Ar–H), 4.12 (m, 1H, $-CH_2-$), 3.98 (m, 1H, $-CH_2-$), 3.40 (d, 1H, −CH−), 2.93 (dd,1H, oxirane), 2.83 (dd,1H, oxirane).^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 44.81, 53.88, 69.85, 114.67, 120.62, 122.81, 130.15, 135.21, 158.65.

(*RS*)-**4**, pale yellow liquid (95% yield), ^{1}H NMR (400 MHz; $CdCl_3$) δ (ppm): 7.64 (dd, 1H, Ar–H), 7.25 (dd, 1H, Ar–H), 7.12 (dd, 1H, Ar–H), 4.11 (dd, 1H, −CH), 4.05 (d, 2H, $-CH_2$), 3.72 (d, 2H, $-CH_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 46.05, 69.67, 69.85,



SCHEME 1 Kinetic resolution of racemic alcohols using various lipases

114.67, 120.62, 122.81, 130.15, 135.21, 158.65. Optimized HPLC method for the estimation of (*RS*)-**4** consisted of Chiralcel OD-H column (0.46-mm diameter, 250-mm long, 5 μm, Chiralcel) with mobile phase Hexane : IPA (90:10), flow rate of 1 mL/min and detected at 254 nm. The retention times for the two enantiomers of (*RS*)-**4** were 9.0 and 10.5 minutes. The retention time for the (*R*)-enantiomer [(*R*)-**4**] was 9.0 minutes. The retention time for the (*R*)-ester [(*R*)-**5**] was 5.6 minutes (Supporting Information S1-S7).

2.3 | Synthesis of (*RS*)-3-(4-chlorophenoxy)propane-1,2-diol [(*RS*)-8]

Chlorphenesin was prepared via chemical route using the following steps.

Chlorphenesin [(*RS*)-**8**] was synthesized by dissolving 4-chlorophenol (**6**) (2.6 g, 20 mM, 1 eqv) in anhydrous acetonitrile followed by the addition of K_2CO_3 (5.2 g, 20 mM, 2 eqv). The reaction mixture was refluxed at 70°C with continuous stirring. After 30 minutes, (*RS*)-glycidol (*RS*)-**7** (2.2 g, 20 mM, 1.5 eqv) was added dropwise. Upon completion of the reaction in 36 hours, the reaction mixture was poured into a mixture of water and dichloromethane, and the organic layer was separated, filtered, dried using anhydrous Na_2SO_4, and concentrated under vacuum. The concentrated crude product was purified using silica gel (60-120 mesh) column chromatography eluted with hexane : ethyl acetate (96:4) affording (*RS*)-**8**. Using similar method enantiopure chlorphenesin, (*S*)-**8** was synthesized.

(*RS*)-**8**, a white solid (90% yield), ^{1}H NMR (400 MHz; $CdCl_3$) δ (ppm): 7.26 (m, 2H, Ar–H), 6.95 (m, 2H, Ar–H), 4.03 (m, 2H, –CH_2), 3.96 (m, 2H, −CH_2), 3.65 (m, 2H, −CH_2), 3.55 (m, 1H, −CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 63.54, 69.44, 70.29, 76.71, 77.03, 77.34, 115.80, 126.24, 129.45, 157.01.

2.4 | Synthesis of (*RS*)-1-chloro-3-(4-chlorophenoxy)propan-2-yl acetate (*RS*)-9

The synthesis of the acetylated derivative of (*RS*)-**8** was done using Ac_2O at 4°C in the presence of pyridine. Using similar method enantiopure-acetylated derivative of chlorphenesin, [(*R*)-**9**] was also synthesized. The optical purity of both the products was determined by chiral HPLC.

(*RS*)-**9**, a white solid (85% yield), ^{1}H NMR (400 MHz; $CdCl_3$) δ (ppm): 7.32 (m, 2H, Ar–H), 6.95 (m, 2H, Ar–H), 4.32 (m, 1H, −CH), 4.27 (m, 2H, −CH_2), 4.12 (m, 2H, −CH_2), 3.32 (m, 1H, −CH), 2.20 (s, 3H, −CH_3). The optimized HPLC method for the estimation of substrate (*RS*)-**8** consisted of Chiralcel AD-H column (0.46-mm diameter, 250-mm long, 5 μm, Chiralcel) with mobile phase Hexane : IPA (90:10) with a flow rate of 1 mL/min and detected at 254 nm. The retention times for the two enantiomers of (*RS*)-**8** were 12.7 and 14.0 minutes. The retention time for (*S*)-**8** was 12.1 minutes. The retention time for the two enantiomers of (*RS*)-**9** were 18.7 and 22.0 minutes. The retention time for (*R*)-ester (*R*)-**9** was 22.0 minutes (Supporting Information S10-S15).

2.5 | Enzyme screening

(*RS*)-**4** and (*RS*)-**8** (0.02 mmol, 1 eqv) were subjected to transesterification with vinyl acetate (acyl donor) (100 μL) in toluene (900 μL) using commercial lipase preparations. All the lipase preparations were individually added and incubated in a shaker at 30°C (200 rpm). After completion, the supernatant was separated and evaporated under vacuum. Samples for HPLC analyses were prepared using 2-propanol, and enantiomeric excess and ratio were determined.

2.6 | Large-scale synthesis of (*S*)-4 and (*S*)-8

Large-scale synthesis of (*S*)-**4** and (*S*)-**8** was performed by subjecting (*RS*)-**4** (511 mg, 2.0 mmol) and (*RS*)-**8** (405 mg,

2.0 mmol), respectively, to transesterification with vinyl acetate (acyl donor) (10 mL) in toluene (90 mL) using *P. fluorescens* lipase. The reaction was incubated in a shaker at 30°C (200 rpm). After completion, the supernatant was separated and evaporated under vacuum. The solvent was evaporated, and crude of the reaction was purified by column chromatography on silica gel (5-20% EtOAc/hexane), affording (*S*)-**4** [49% isolated yield and >95% ee], (*R*)-**5** [48% isolated yield and >95% ee], and (*S*)-**8** [49% isolated yield and >95% ee], (*R*)-**9** [48% isolated yield and >95% ee].

2.7 | Optimization of process parameters for the enantioselective transesterification of (*RS*)-4 and (*RS*)-8

Effect of each parameter influencing the lipase-catalyzed kinetic resolution was studied one at a time. Six organic solvents with different log *P* values were used to study the effect on the transesterification of (*RS*)-**4**. The optimum reaction time was determined by performing the reaction and collecting the samples at various time intervals. Various aspects of enzymatic acylation such as enantioselectivity, conversion, and greenness have been reported to be influenced by the acyl donors. The enantioselectivity and rate of conversion of enzyme-catalyzed kinetic resolution was therefore studied using various acyl donors. The reaction temperature also influences the rate and enantioselectivity of the transesterification reaction. Various enzyme concentrations (15, 30, 45, 60, and 90 mg/mL) were used with a fixed substrate concentration (20 mM). Substrate concentrations were also varied (10, 20, 30, 40, and 50 mM) to achieve its optimum concentration for the reaction. Samples were analyzed for conversion and enantioselectivity of the enzymes used.

2.8 | Methodology for molecular docking

Glide module of Schrödinger software incorporated with the maestro graphical user interface (GUI) was used for molecular docking study. Initially, appropriate 3D crystal structures for the lipase *Pseudomonas* sp. MIS38 (selected on the basis of similarity with PFL) was retrieved from the Protein Data Bank (PDB). The lipase was prepared using protein preparation wizard incorporated in Schrodinger software. The bond orders for the protein residues were assigned, and hydrogen atoms, which were not assigned for each amino acid in the 3D crystal structure, were added. The disulfide bonds were created, and appropriate crystal water molecules, which were part of active site, were considered during molecular docking process. Finally, lipase structure was minimized using incorporated OPLS_2005 force field. The grid for the protein was generated using the centroid of the bound ligand as well as using selective active site residues mentioned in the literature. The MOL files of ligand structures were opened and prepared using LigPrep module of Schrödinger software, and possible ionization states were generated at neutral pH (7.0 ± 0.5). Finally, prepared ligands were docked in the active site of the lipase using the standard precision method from the glide module of Schrodinger software, and 30 poses per ligand were generated. The best docked conformations (poses) were selected on the basis of G score and interactions.

3 | RESULTS AND DISCUSSION

Lipases follow the well-known "ping-pong bi-bi" mechanism of enantioselective transesterification.[10] Enantioselectivity is obtained because of the differential interaction of *R*- and *S*-enantiomers with catalytically active site of the lipase.[39,41] Lipases are well known for the kinetic resolution of secondary alcohols with a stereochemical preference toward the *R*-enantiomer.[20]

3.1 | Lipase-catalyzed transesterification of (*RS*)-4 and (*RS*)-8

(*RS*)-**4** and (*RS*)-**8** were synthesized and subjected to lipase-catalyzed kinetic resolution (Scheme 1). Five different commercially available lipases (*Candida rugosa* [CRL], *Burkholderia cepacia* [BCL], *P. fluorescens* [PFL], *Candida antarctica* [CALA], and *Candida antarctica* [CALB]) were screened for the kinetic resolution of both (*RS*)-**4** and (*RS*)-**8** in toluene using vinyl acetate as acyl donor. No reaction was observed in the absence of lipase. Among all the lipases screened, *P. fluorescens* lipase (PFL) was the fastest enzyme to resolve both (*RS*)-**4** and (*RS*)-**8** into the corresponding (*S*)-alcohol and (*R*)-ester products, with high enantioselectivity. Other lipases also performed the enantioselective transesterification of (*RS*)-**4** and (*RS*)-**8**, but with poor conversion. Thus, PFL was chosen for further reactions. The most interesting outcome of this study was the rapid PFL-catalyzed enantioselective conversion of the racemates compared to other lipases (Tables S1 and S2).

3.2 | Effect of solvents

Reaction medium plays an important role in deciding the course of a biocatalytic reaction. Study of lipase catalysis in organic solvents is very interesting and well researched.[22,45] Lipases are interfacial enzymes, and the enzyme-bound water (called as structural water, generally

present in the form of a monolayer) plays a crucial role for the enzyme structure and is necessary for their catalytic activity.[18] Hydrophilic solvents pose a higher tendency to strip the tightly bound water decreasing the efficiency of the lipase. Contrary to this, lipases exhibit good stability in organic solvents, which have a lesser tendency to do so, thus becoming a favorable reaction medium for lipase-catalyzed reactions.[37] Many examples of improved lipase activity and selectivity in organic solvents along with other benefits like increased solubility of substrates have been reported.[19,22] In the same context, different organic solvents, varying in log *P* values were examined for the kinetic resolution of (*RS*)-**4** and (*RS*)-**8**, with vinyl acetate using PFL. In the case of (*RS*) **4**, best result was obtained in hexane depicting 50.7% conversion, 97.1% ee_s, 94.6% ee_p, and enantiomeric ratio (E-value) of 153 (Figure 1A). Similarly, toluene gave maximum enantiomeric excess and enantioselectivity for (*RS*)-**8** with 50.4% conversion, 96.3% ee_s, 95.0% ee_p, and enantiomeric ratio (E-value) of 152 (Figure 2A).

3.3 | Type of acyl donors

Lipase-catalyzed acyl transfer reactions involving normal esters are generally reversible and yield low optical purity of the product and substrate. The use of enol esters like vinyl acetate or isopropenyl acetate shifts the reaction equilibrium toward the product.[33] These esters lead to the liberation of unstable enols, which are rapidly tautomerized to aldehydes and ketones, thereby making the reaction irreversible in nature.[9,29] However, the acetaldehyde formed during the reaction might act as an alkylating agent and deactivate the lipase. This is not a general case with all the lipases and the degree of deactivation depends upon the nature of the lipase.[11] Hence, the type of acyl donor and interaction with the lipase is a pivotal determinant of reaction progression. Four different acyl donors (BA, benzyl acetate; EA, ethyl acetate; IPA, isopropenyl acetate; VA, vinyl acetate) were applied in the reaction, among which vinyl acetate showed best results in the kinetic resolution of both (*RS*)-**4** and (*RS*)-

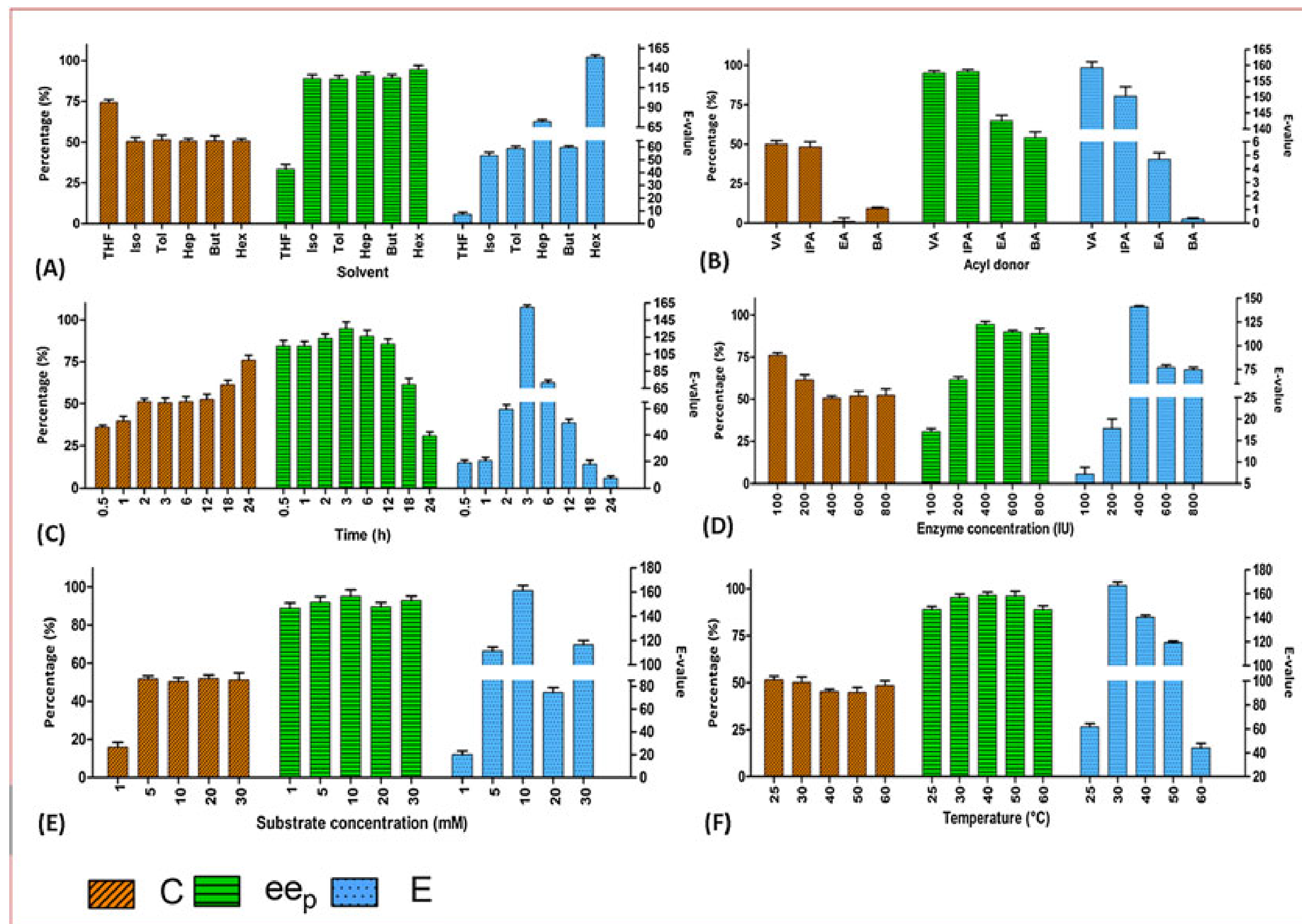


FIGURE 1 Effect of (A) solvents, (B) acyl donors [BA = benzyl acetate, EA = ethyl acetate, IPA = isopropenyl acetate, VA = vinyl acetate], (C) reaction time, (D) substrate concentration, (E) enzyme concentration, and (F) temperature on PFL-catalyzed kinetic resolution of [(*RS*)-**4**]

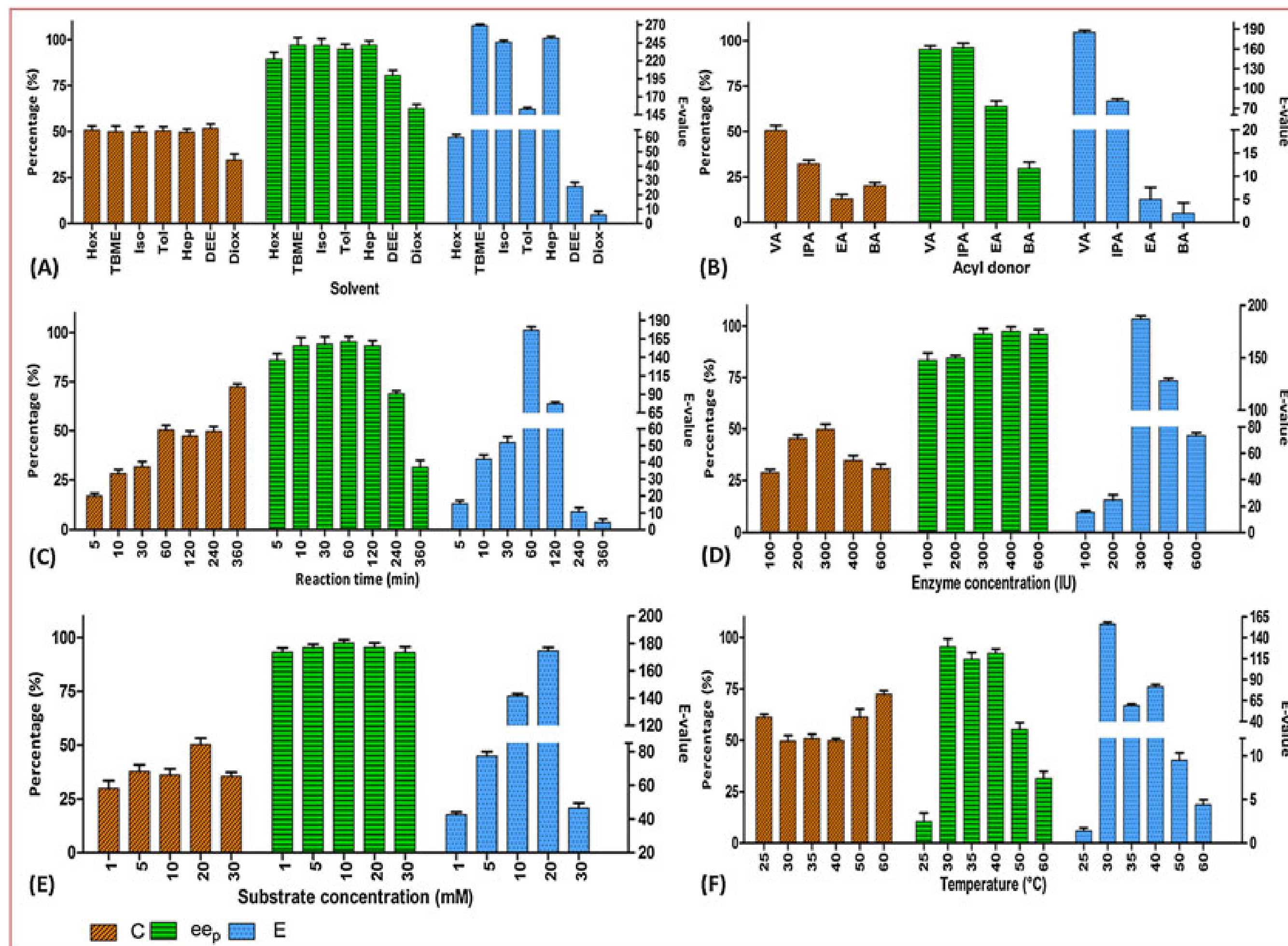


FIGURE 2 Effect of (A) solvents, (B) acyl donors, (C) reaction time, (D) substrate concentration, (E) enzyme concentration, and (F) temperature on the PFL-catalyzed kinetic resolution of [(*RS*)-**8**]

8. The highest percentage conversion (50.1%), enantiomeric excess (95.7% ee_s and 95.2% ee_p) and E-value of 159 for PFL-catalyzed kinetic resolution of (*RS*)-**4** in hexane were obtained with vinyl acetate as an acyl donor (Figure 1B). Similarly for (*RS*)-**8**, highest percentage conversion (50.6%), enantiomeric excess (97.7% ee_s and 95.3% ee_p), and E-value of 185 were obtained in PFL-catalyzed kinetic resolution using vinyl acetate (Figure 2B).

3.4 | Optimization of reaction time

The most important factor governing the utility of a biocatalytic process is reaction time. The industrial research is focused on developing economically expedient processes, yielding higher enantioselectivity in lesser time. Gratifyingly, in the present study PFL catalyzed the kinetic resolution of both (*RS*)-**4** and (*RS*)-**8** in a short duration of 3 and 1 hour, respectively. In the reaction of (*RS*)-**4**, PFL took 3 hours to give 97.1% ee_s, 94.8% ee_p, 50.6% conversion, and E-value 160 (Figure 1C). Likewise, for (*RS*)-**8**, the optimum reaction time was 1 hour with 96.8% ee_s, 95.4% ee_p, 50.4% conversion, and E-value 177 (Figure 2C). Other lipases also give moderate-to-good results but in longer duration, making PFL the obvious choice for this biocatalytic process. The prompt activity of PFL could be due to better accommodation of a particular conformation of the substrate at the active site of PFL. The molecular docking study in the later section is a demonstrative evidence of such interactions. The swift action of PFL with high degree of enantioselectivity and rate of conversion makes it a very promising tool in such reactions.

3.5 | Optimization of substrate and enzyme concentration

Lipases are interfacial enzymes. It is a unique characteristic of lipases. Several studies have revealed the effect of the interfacial microenvironment in lipase catalysis. Thus, it is important to determine the correlation of lipase

activity with the concentration of enzymes and substrates.[31] The hydrophobic and electrostatic interactions between the enzyme and substrate at the interface are important with respect to the diffusion of substrates toward the active site. At low interfacial concentration of the substrate, lipase exhibits little activity. However, once the substrate concentration reaches an optimum value (exceeding its solubility limit), and the substrate becomes available in the form of micelles, the reaction rate increases.[36] In the light of the aforementioned explanations, we performed the reaction using a range of substrate concentration. The substrate concentration was varied between 1 and 30 mM. In case of (*RS*)-**4** the optimal substrate concentration was 10 mM, at which PFL displayed good conversion rate of 50.4% and E-value of 161 along with the ee_s and ee_p values of 96.5% and 95.1%, respectively (Figure 1E). Similarly for (*RS*)-**8** the optimal substrate concentration was 20 mM at which the percentage conversion was 50.3, E-value of 175 and ee_s and ee_p were found to be 96.4% and 95.5%, respectively (Figure 2E). The rate of an enzymatic reaction depends upon the amount of the enzyme present in the reaction medium, which in turn is an indicator of the number of enzyme active sites of the enzyme. Generally, rate of the reaction increases with the increase in enzyme concentration. However, at an optimum concentration of the enzyme all the active sites of the enzyme are saturated, and further increase in the enzyme concentration does not improve the reaction rate. Thus, an optimum concentration of enzyme is sought for high yield, less reaction time, and economic viability of the process.[21] The reactions of (*RS*)-**4** and (*RS*)-**8** were performed using different concentrations (100, 200, 300, 400, 600, and 800 IU/mL) of PFL in hexane and toluene, respectively. At 400 IU/mL, PFL showed highest conversion of 50.6%, E-value of 141 and ee_s and ee_p were found to be 96.6% and 94.4%, respectively for (*RS*)-**4** (Figure 1D). For (*RS*)-**8**, while the maximum conversion with 300 IU/mL PFL was 49.7%, E-value of 187 and ee_s and ee_p were found to be 95.0% and 96.1%, respectively (Figure 2D).

3.6 | Optimization of reaction temperature

The value of enantioselectivity (E-value) in a lipase-catalyzed reaction depends upon the difference in the free energy of activation (G) of the two enantiomers, which in turn is related to the activation enthalpy (H) and the activation entropy (S), as $G = H - TS$. The difference in activation enthalpy of the enantiomers is due to the difference in the steric fit of the substituents into the respective binding pockets of the lipase. If the values of H and TS are balanced then temperature can modulate the stereochemistry of the reaction. While some studies suggest that enantioselectivity increases upon lowering the temperature, many other studies depict an increase in enantioselectivity with the rise in temperature. Thus, change in temperature has a variable effect on lipase-catalyzed reactions.[32,35] The kinetic resolution of both the racemates was performed at a range of temperature (25, 30, 40, 50, and 60°C) to observe the effect on degree of enantioselectivity and percentage conversion. In both the reactions of (*RS*)-**4** and (*RS*)-**8**, PFL catalyzed the conversion with highest enantioselectivity at 30°C. The increase in temperature showed a gradual demise in the enantioselective resolution of the (*R*) and (*S*) forms of the racemic alcohols. For (*RS*)-**4** the conversion at 30°C was 50.2%, with 96.0% ee_s, 95.4% ee_p, and an E-value of 167 after 3 hours of reaction (Figure 1F). For (*RS*)-**8** the conversion at 30°C was 49.6%, with 94.0% ee_s, 95.5% ee_p, and an E-value of 156 after 1 hour of reaction (Figure 2 F; Supporting Information S9 and S17).

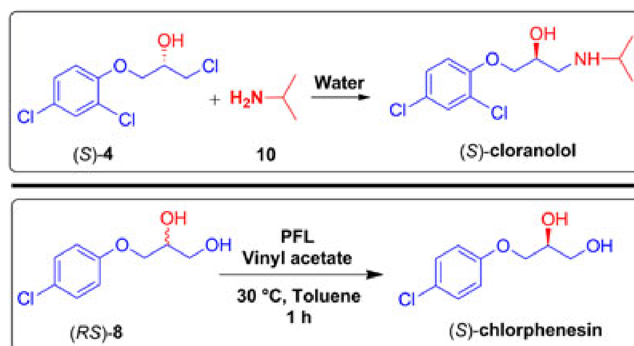
3.7 | Molecular docking study for the validation of suitable biocatalyst

Molecular docking of substrates into the active site of the enzyme gives an insight of the reaction mechanism. The crystal structures of many lipases with small molecules bound at the active site are well exploited in the study of catalytic mechanism and enantioselectivity of biocatalytic reactions.[12,14] In our investigation of theoretical evidence for the experimental work, we performed molecular docking of the *R*- and *S*-enantiomers of (*RS*)-**4** and (*RS*)-**8**, using Schrodinger's Maestro Glide 9.2 program. Wet laboratory experiments revealed PFL as the most suitable lipase for both (*RS*)-**4** and (*RS*)-**8**. While searching the PDB [www.rcsb.org], we discovered that the 3D crystal structure information of PFL has not hitherto been reported. A protein sequence annotated as lipase from *P. fluorescens* (P41773 LIPB_PSEFL: 447 AA) was available in the UniProt database. BLAST analysis of this sequence exhibited the highest percent identity (79%) and query cover (99%) with *Pseudomonas* sp. MIS38 (PDB code 2Z8X).[1] On the basis of the similarity score *Pseudomonas* sp. MIS38 was used for docking of the 4 enantiomers. Crystal structure of the lipase displayed catalytic triad composed of Ser207, Asp255, and His313 residues and the oxyanion hole containing the –NH groups of the peptide backbone constituted by Tyr29, Gly59, and Arg106. According to the well-known "ping-pong bi-bi" mechanism, the alcohol does not act on the apoenzyme but on the acyl enzyme intermediate. Hydrogen bonds between the carbonyl O atom of the acyl group and the –NH groups of the oxyanion hole stabilize the acyl enzyme complex. Further, a nucleophilic attack by the

alcohol leads to the formation of an ester.[17] To explore molecular docking for evaluation of binding differences between the enantiomers, we prepared the acyl enzyme intermediate by acylation of the hydroxyl group of serine 207. In case of (*RS*)-**4**, the *R*-enantiomer, ie, (*R*)-**4** showed strong H-bond interaction with Tyr29 and His 313 each with a minimum distance of 1.9 Å (Figure 3A). The (*S*)-enantiomer showed lesser interactions needed for the lipase-catalyzed reaction (Figure 3B). The G score for the *R*-enantiomer was −5.67, while the *S*-enantiomer had a lower score of −5.24. It thus indicated that PFL catalyzed the transesterification of both the enantiomers, with preference to the *R* form. However, this selectivity was time dependent. Initially, PFL catalyzed the reaction very fast in favor of transesterification of the *R*-enantiomer. Maximum conversion of (*RS*)-**4** into the corresponding ester (*R*)-**5** was achieved in 3 hours. The result obtained in 3 hours was C = 50.6%, ee_S = 97.1%, ee_P = 94.8%, and E = 160. Beyond this period, lipase catalyzed nonselective conversion of (*RS*)-**4**, thereby losing the enantioselectivity. Similarly, in the case of (*RS*)-**8**, the *R*-enantiomer, ie, (*R*)-**8** showed strong interactions with Tyr29 and Gly59 residue each with an H-bond distance of 1.9 and 2.6 Å (Figure 3 C). The *S*-enantiomer showed lesser interaction (Figure 3D). The G score for the *R*-enantiomer was −5.36, while the *S*-enantiomer had a lower score of −4.13. PFL catalyzed the maximum conversion of (*RS*)-**8** into (*R*)-**9** in a very short duration of 1 hour. The result obtained in 1 hour was C = 50.4%, ee_S = 96.8%, ee_P = 95.4%, and E = 177. Other lipases also performed the enantioselective transesterification of (*RS*)-**4** and (*RS*)-**8**, but best results were obtained with PFL, the most interesting feature being the least time taken by PFL compared to other lipases.

3.8 | Synthesis of the enantiopure drugs using the results of computational and experimental studies

The results obtained from the initial screening of the biocatalyst and optimization of the reaction parameters were incorporated in the synthesis of enantiopure cloranolol and chlorphenesin. (*S*)-**4** was successfully synthesized by the kinetic resolution of (*RS*)-**4** using *P. fluorescens* lipase with C = 50.2%, E = 161, ee_p = 96.0%, and ee_s = 95.2%.



SCHEME 2 Synthesis of final drugs

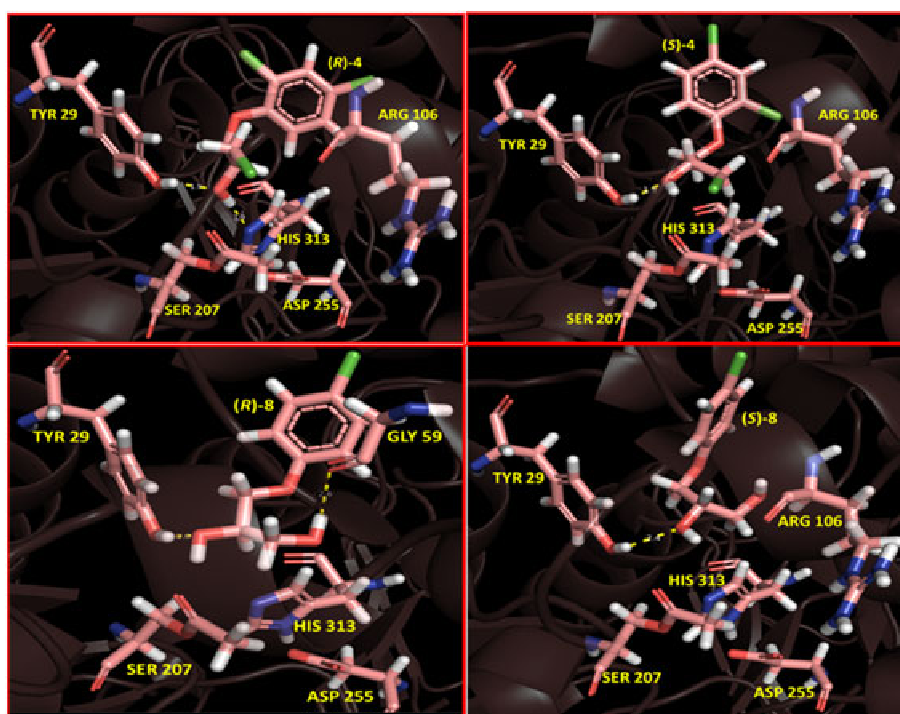


FIGURE 3 Interaction of ligands (A) [(*R*)-4], (B) [(*S*)-4], (C) [(*R*)-8], and (D) [(*S*)-8] with *Pseudomonas* sp. MIS38 lipase

(*S*)-**4** was further reacted with isopropyl amine (**10**) in water to produce (*S*)-cloranolol (89% yield).

Enantiopure chlorphenesin, (*S*)-**8** was obtained by the kinetic resolution of (*RS*)-**8** using *P. fluorescens* lipase (Scheme 2).

4 | CONCLUSION

Our investigation was focused on the screening of lipases for kinetic resolution of two useful racemates. Central to this investigation was the observation that *P. fluorescens* lipase catalyzed the kinetic resolution expeditiously taking only 3 and 1 hour for the two racemates, respectively. Under optimized conditions, PFL exhibited selective acylation of the (*R*) enantiomer of (*RS*)-**4** [C = 50.2%, ee_s = 96.0%, ee_p = 95.2%, and E = 161] and (*RS*)-**8** [C = 50.3%, ee_s = 96.2%, ee_p = 95.0%, E = 153]. To our delight, molecular docking results displayed considerable interaction between the lipase and *R*-enantiomers of the racemates, which was in agreement with experimental results. The methodology of complementing biocatalytic reactions with molecular docking proved to be a time-efficient and reliable process. The result of both studies taken together warranties a broad application of *P. fluorescens* lipase as a biocatalyst for the synthesis of enantiopure intermediates.

ACKNOWLEDGMENTS

S.S. would like to thank the Department of Science and Technology, Government of India, for providing DST-INSPIRE fellowship. B.P.D. and V.K.S. acknowledge NIPER, Mohali, for providing fellowship. G.P. acknowledges the Department of Biotechnology, Government of India, for providing DBT-fellowship. Authors acknowledge the Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research, S. A.S. Nagar 160062, Punjab, India, for providing facilities for docking study.

ORCID

Uttam C. Banerjee http://orcid.org/0000-0002-7363-4042

REFERENCES

1. https://blast.ncbi.nlm.nih.gov/Blast.cgi.
2. Agustian J, Kamaruddin AH, Aboul-Enein HY. Enantio-conversion and selectivity of racemic atenolol kinetic resolution using free *Pseudomonas fluorescence* lipase (amano) conducted via transesterification reaction. *RSC Adv*. 2016;6:26077-26085.
3. Banoth L, Thakur NS, Bhaumik J, Banerjee UC. Biocatalytic approach for the synthesis of enantiopure acebutolol as a β_1-selective blocker. *Chirality*. 2015;27:382-391.
4. Bonrath W, Karge R, Netscher T. Lipase catalyzed transformations as key steps in the large scale preparation of vitamins. *J Mol Catal B: Enzym*. 2002;19–20:67-72.
5. Brem J, Turcu MC, Paizs C, et al. Immobilization to improve the properties of *Pseudomonas fluorescens* lipase for the kinetic resolution of 3-aryl-3-hydroxy esters. *Process Biochem*. 2012;47:119-126.
6. Caro Y, Masaguer CF, Ravina E. Preparation of (*R*)-(−)- and (*S*)-(+)-3-Hydroxymethyl-1-tetralone tosylates, key intermediates in the synthesis of new CNS drugs via resolution of precursors. *Tetrahedron Asymmetry*. 2003;14:381-387.
7. Dwivedee BP, Ghosh S, Bhaumik J, Banoth L, Banerjee UC. Lipase-catalyzed green synthesis of enantiopure atenolol. *RSC Adv*. 2015;5:15850-15860.
8. Dwivedee BP, Bhaumik J, Rai SK, Laha JK, Banerjee UC. Development of nanobiocatalysts through the immobilization of *Pseudomonas fluorescens* lipase for applications in efficient kinetic resolution of racemic compounds. *Bioresour Technol*. 2017;239:464-471.
9. Egri G, Baitz-Gács E, Poppe L. Kinetic resolution of 2-acylated-1,2-diols by lipase-catalyzed enantiomer selective acylation. *Tetrahedron Asymmetry*. 1996;7:1437-1448.
10. Ema T. Mechanism of enantioselectivity of lipases and other synthetically useful hydrolases. *Curr Org Chem*. 2004;8:1009-1025.
11. Faber K, Riva S. Enzyme-catalyzed irreversible acyl transfer. *Synthesis (Stuttg)*. 1992;10:895-910.
12. Ferrario V, Ebert C, Knapic L, et al. Conformational changes of lipases in aqueous media: a comparative computational study and experimental implications. *Adv Synth Catal*. 2011;353:2466-2480.
13. Ferreira HV, Rocha LC, Severino RP, Porto ALM. Syntheses of enantiopure aliphatic secondary alcohols and acetates by bioresolution with lipase B from *Candida antarctica*. *Molecules*. 2012;17:8955-8967.
14. García-Urdiales E, Busto E, Ríos-Lombardía N, Gotor-Fernández V, Gotor V. Computational study of the lipase-mediated desymmetrisation of 2-substituted-propane-1,3-diamines. *Chembiochem*. 2009;10:2875-2883.
15. Gotor V. Biocatalysis applied to the preparation of pharmaceuticals. *Org Process Res Dev*. 2002;6:420-426.
16. Gotor-Fernández V, Brieva R, Gotor V. Lipases: useful biocatalysts for the preparation of pharmaceuticals. *J Mol Catal B: Enzym*. 2006;40:111-120.
17. Guieysse D, Cortés J, Puech-Guenot S, et al. A structure-controlled investigation of lipase enantioselectivity by a path-planning approach. *Chembiochem*. 2008;9:1308-1317.
18. Hari Krishna S, Karanth NG. Lipases and lipase-catalyzed esterification reactions in nonaqueous media. *Cata Rev*. 2002;44:161-4940.
19. Herbst D, Peper S, Niemeyer B. Enzyme catalysis in organic solvents: influence of water content, solvent composition and

temperature on candida rugosa lipase catalyzed transesterification. *J Biotechnol*. 2012;162:398-403.

20. Höhne M, Schätzle S, Jochens H, Robins K, Bornscheuer UT. Rational assignment of key motifs for function guides in silico enzyme identification. *Nat Chem Bio*. 2010;6:807-813.
21. Janes LE, Kazlauskas RJ, Quick E. A fast spectrophotometric method to measure the enantioselectivity of hydrolases. *J Org Chem*. 1997;3:4560-4561.
22. Klibanov AM. Improving enzymes by using them in organic solvents. *Nature*. 2001;409:9-11.
23. Kulcsár-Gergely J. Action of beta-adrenoceptor blockers on liver function of rats. *Arzneimittelforschung*. 1989;39:782-785.
24. Łukowska-Chojnacka E, Staniszewska M, Bondaryk M, Maurin JK, Bretner M. Lipase-catalyzed kinetic resolution of novel antifungal N-substituted benzimidazole derivatives. *Chirality*. 2016;28:347-354.
25. Margolin AL. Enzymes in the synthesis of chiral drugs. *Enzyme Microb Technol*. 1993;15:266-280.
26. Muñoz Solano D, Hoyos P, Hernáiz MJ, Alcántara AR, Sánchez-Montero JM. Industrial biotransformations in the synthesis of building blocks leading to enantiopure drugs. *Bioresour Technol*. 2012;115:196-207.
27. Newman DJ. Natural product-derived drugs based on β-adrenergic agents and nucleosides. *J Braz Chem Soc*. 2016;27:1320-1333.
28. Panchgalle SP, Gore RG, Chavan SP, Kalkote UR. Organocatalytic enantioselective synthesis of β-blockers: (*S*)-Propranolol and (*S*)-Naftopidil. *Tetrahedron Asymmetry*. 2009;20:1767-1770.
29. Paravidino M, Hanefeld U. Enzymatic acylation: assessing the greenness of different acyl donors. *Green Chem*. 2011;13:2651-2657.
30. Pchelka BK, Loupy A, Plenkiewicz J, Petit A, Blanco L. Resolution of racemic 3-aryloxy-1-nitrooxypropan-2-ols by lipase-catalyzed enantioselective acetylation. *Tetrahedron Asymmetry*. 2001;12:2109-2119.
31. Petersen MTN, Fojan P, Petersen SB. How do lipases and esterases work: the electrostatic contribution. *J Biotechnol*. 2001;85:115-147.
32. Phillips RS. Temperature modulation of the stereochemistry of enzymatic catalysis: prospects for exploitation. *Trends Biotechnol*. 1996;14:13-16.
33. Puskas JE, Seo KS, Sen MY. Green polymer chemistry: precision synthesis of novel multifunctional poly(ethylene glycol)s using enzymatic catalysis. *Eur Polym J*. 2011;47:524-534.
34. Quan J, Wu Q, Lin XF. Synthesis of polymeric prodrugs of chlorphenesin with saccharide branches by chemo-enzymatic regioselective strategy. *Polymer (Guildf)*. 2007;48:2595-2604.
35. Reetz MT. Lipases as practical biocatalysts. *Curr Opin Chem Biol*. 2002;6:145-150.
36. Reis P, Holmberg K, Watzke H, Leser ME, Miller R. Lipases at interfaces: a review. *Adv Colloid Interface Sci*. 2009;147–148:237-250.
37. Salihu A, Alam MZ. Solvent tolerant lipases: a review. *Process Biochem*. 2015;50:86-96.
38. Salis A, Bhattacharyya MS, Monduzzi M, Solinas V. Role of the support surface on the loading and the activity of *Pseudomonas fluorescens* lipase used for biodiesel synthesis. *J Mol Catal B: Enzym*. 2009;57:262-269.
39. Sawant G, Ghosh S, Banesh S, Bhaumik J, Banerjee UC. In-silico approach towards lipase mediated chemoenzymatic synthesis of (*S*)-ranolazine as an antianginal drug. *RSC Adv*. 2016;6:49150-49157.
40. Syrenski AV, Tsyrlin VA. The effect of beta-adrenoblockaders on the elasticity of the vessels of the greater circulation. *Eksp Klin Farmakol*. 1995;58:23-24.
41. Thakur NS, Bhaumik J, Sooram B, Banoth L, Banerjee UC. Synthesis of enantiopure drugs and drug intermediates using in silico generated archetype biocatalyst: a case study using alprenolol as a model drug. *ChemistrySelect*. 2016;1:871-876.
42. Theil F, Ballschuh S, Kunath A, Schick H. Kinetic resolution of rac-3-(2-methylphenoxy)propane-1,2-diol (mephenesin) by sequential lipase-catalyzed transesterification. *Tetrahedron Asymmetry*. 1991;2:1031-1034.
43. Wang ZM, Zhang XL, Sharpless KB. Asymmetric dihydroxylation of aryl allyl ethers. *Tetrahedron Lett*. 1993;34:2267-2270.
44. Wang F, Zhang H, Wang J, et al. Ultrasound irradiation promoted enzymatic transesterification of (*R*/*S*)-1-Chloro-3-(1-naphthyloxy)-2-propanol. *Molecules*. 2012;17:10864-10874.
45. Zaks A, Klibanov AM. Enzymatic catalysis in nonaqueous solvents. *J Biol Chem*. 1988;263:3194-3201.
46. Zhang JY, Liu HM, Xu HW, Shan LH. Non-enzymatic kinetic resolution of β-amino alcohols using C-12 higher carbon sugar as a chiral auxiliary. *Tetrahedron Asymmetry*. 2008;19:512-517.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Soni S, Dwivedee BP, Sharma VK, Patel G, Banerjee UC. Exploration of the expeditious potential of *Pseudomonas fluorescens* lipase in the kinetic resolution of racemic intermediates and its validation through molecular docking. *Chirality*. 2017;1–10. https://doi.org/10.1002/chir.22771