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In-silico approach towards lipase mediated chemoenzymatic synthesis of (*S*)-Ranolazine, as an anti-anginal drug

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In silico modelling based biocatalytic approach for the synthesis of drugs and drug intermediates in enantiopure forms is a rationalized methodology over the organo-chemical routes. In this study, enzyme-ligand based docking was carried out using (*RS*)-ranolazine, as the model drug for the screening of a suitable biocatalyst for the kinetic resolution of the racemic drug. The differential interaction of the two enantiomers with the lipase was analyzed on the basis of docking score and H-bond interaction with the amino acid residues, which helped to define the trans-esterification mechanism. Ranolazine [*N*-(2,6-dimethylphenyl)-2-[4-(2-hydroxy-3-(2-methoxyphenoxy)propylpiperazin-1-yl]acetamide], an anti-anginal drug, significantly reduces the frequency of anginal attack and has also been used for the treatment of ventricular arrhythmias, and bradycardia. Various lipases were examined via computational as well as wet lab screening and *Candida antartica* lipase in the form of CLEA was the most efficient one for the (*S*)-selective kinetic resolution of (*RS*)-Ranolazine, with highest conversion and enantiomeric excess. This is the first report of chemo-enzymatic synthesis of (*S*)-Ranolazine where the whole drug molecule was used for lipase catalysis. The present study showed that the combination of *in silico* studies and classical wet lab approach could change the paradigm of biocatalysis.

Keywords: In silico, Biocatalysis, Ranolazine, Lipase, Enantiopure, Chemo-enzymatic

1. Introduction

Computational approach for the exploration of suitable biocatalyst candidates for the enantioselective synthesis of drugs and drug intermediates is an emerging field in chemoenzymatic synthesis[1]. Docking of small molecules with the enzymes/receptors reveals the information about the binding nature and interactions with the enzyme residues. In chiral compound synthesis, enantioselectivity of the biocatalyst (enzyme) is a crucial factor and screening of catalysts in terms of selectivity is also a major concern[2]. The combination of enzyme kinetics and molecular modelling was used to construct a model of how the enantiomers of secondary alcohols bind in the active site of this enzyme during transesterfication reactions[3]. The two enantiomers of a secondary alcohol are shown to bind in two different orientations in the active site. The enantioselectivity is caused by the difference in the free energy gaps between the ground states of the enantiomers and the corresponding transition states, which the enantiomers form with the enzyme.

The thermodynamic ground states of the enantiomers are the same. Therefore, the enantioselectivity is determined by the difference in free energy between the two transition states[4].

For small molecule docking, various softwares are available such as GOLD, MOE, Molegro Virtual Docking, Maestro Glide and each one of them has different scoring algorithm[5]. According to various reports, biocatalysts are used in most of the biotransformation reactions with high regio- and stereoselectivity[6-9]. Enzymes are environment friendly alternatives over conventional chemical catalysts as they can operate under ambient temperature and pressure[10,11]. In pharmaceutical sector, the new biocatalytic approaches for the synthesis of drugs and drug intermediates are particularly appealing due to low cost and minimal use of toxic chemicals[8,12,13,14.] Lipases belonging to the class of serine hydrolases (α/β hydrolases super family) can act on the organic–aqueous interface and their industrial potential is also well known[15,16,17]. Notably, the mechanism of transesterification reaction of lipase through catalytic triad has been already described by various researchers[18,19]. Incorporation of *in silico* studies along with the conventional biocatalytic approach reduces both time and production expenses significantly. It will help to select suitable enzyme preparations prior to the wet laboratory screening and importantly will give access to determine the mechanism of biocatalysis.

Ranolazine has been approved by US FDA in 2006[20] for the treatment of chronic angina as it significantly reduces frequent anginal attack and replaces the use of short acting nitroglycerine[21]. According to the report of the American Heart

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Association, cardiovascular disease is the leading cause of global mortality, accounting for 17.3 million deaths per year, which is expected to reach to more than 23.6 million by 2030[22]. In this study, Ranolazine [*N*-(2, 6-dimethylphenyl)-2-[4-(2-hydroxy-3-(2-methoxyphenoxy)propyl)piperazin-1-yl] acetamide], member of the ß blocker family used as an anti-anginal agent[23] was selected as the model drug for the computational catalysis. Unlike other drugs used to treat chronic angina, ranolazine shows negligible effect on heart rate and blood pressure. The major mechanism is believed to be its inhibitory effect on late sodium influx during the cardiac potential of cardiomyocytes, which is particularly important in ischemic or hypoxic condition[24]. By altering the intracellular sodium level, ranolazine can affect the sodium-dependent calcium channels during myocardial ischemia. Thus, ranolazine indirectly prevents the calcium overload and prevent cardiac ischemia[25]. Of the two enantiomers, (*S*)-enantiomer showed the anti-anginal activity whereas the (*R*)-form showed anti-diabetic activity[26]. The (*S*)-enantiomer is potentially stronger and better anti-arrhythmic drug than either the (*R*)-enantiomer or racemic ranolazine[26]. Several chemical methods were reported for the synthesis of (*RS*)-ranolazine or enantiomers. A synthetic procedure continuing a single step was reported by Li et al. (2003) for the synthesis of (*RS*)-ranolazine with 96% yield[27]. In another report, one step synthesis was described with 90% yield (Lu et al., 2004)[28]. An improved process for the synthesis of (*RS*)-ranolazine with 68% yield was reported by Aalla *et al*[29]. These chemical synthetic methods involve costly catalysts and harsh reaction conditions, therefore they are neither cost-effective nor environment friendly. Biocatalysis can be the only alternative to make the process benign and economical. The first chemoenzymatic synthesis of both enantiomers of ranolazine was reported by Moen *et al*. (2005)[30]. A multistep synthetic procedure of ranolazine was also reported with 70% yield[31]. There was a report on water based chemistry for the synthesis of (*RS*)/(*R*)/(*S*)-ranolazine by Kommi et al. (2013) with 93% yield[32]. Regioselective ring opening by tangstate mediated catalysis for the synthesis of ranolazine was also reported by Pathare et al. (2013) with 85% yield[33].

Here we report a retrosynthetic pathway for (*S*)-ranolazine as shown in Figure 1. Lipase mediated kinetic resolution of the entire drug (also synthesized chemically) was carried out. This is the first instance where the complete drug has been used for the resolution. In-silico approach has been used for the screening of lipases and to determine the enantioselective reaction mechanism. Overall, a combination of in silico and biocatalytic approach afforded the enantiopure ranolazine as a breakthrough in the treatment of cardiovascular diseases.

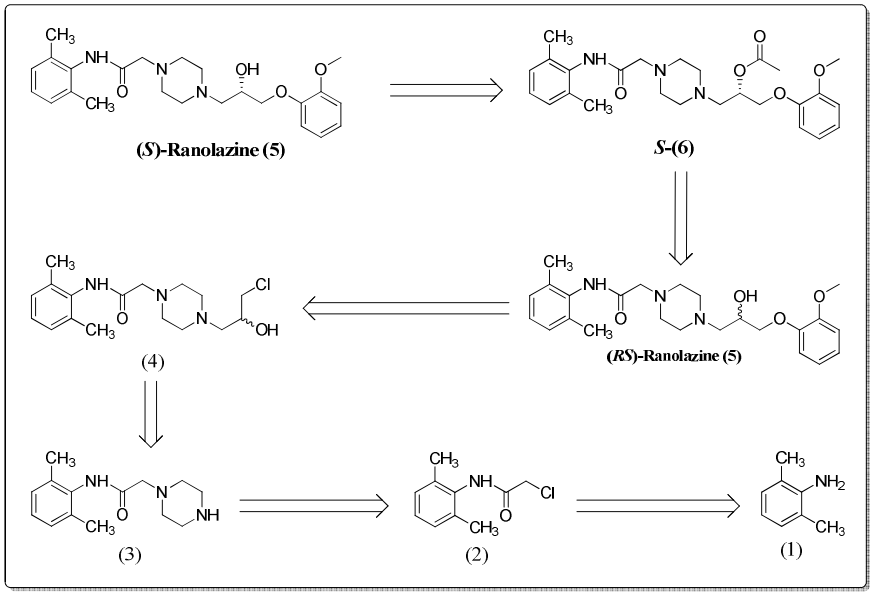

Figure 1. Retrosynthetic pathway for the synthesis of (S)-ranolazine

2. Results and Discussion

2.1 Chemical synthesis of (*RS*)-ranolazine as a standard compound

Prior to optimizing and synthesizing the enatiopure ranolazine through biocatalytic route, it was necessary to prepare the standard compound via a chemical route. Recently a research group has developed a chemical route for the synthesis of enatiopure ranolazine. However, the major pitfall of the method was the use of expensive catalysts.[32] We modified the chemical pathway to synthesize racemic ranolazine as described below.

The first building block of ranolazine synthesis, 2-chloro-*N*-(2,6-dimethylphenyl) acetamide **2** was synthesized by the reaction of 2,6-dimethylaniline **1** and chloroacetylchloride with 97% yield (Scheme 1). Further, to incorporate the piperazine ring, compound **2** was treated with piperazine which resulted into the formation of product **3** with 70% yield (Scheme 1). To introduce the chiral center, *N*-(2,6-dimethylphenyl)-2-(piperazin-1-yl)acetamide **3** was treated with (*RS*)-epichlorohydrin in the presence of SDS in water as a solvent which

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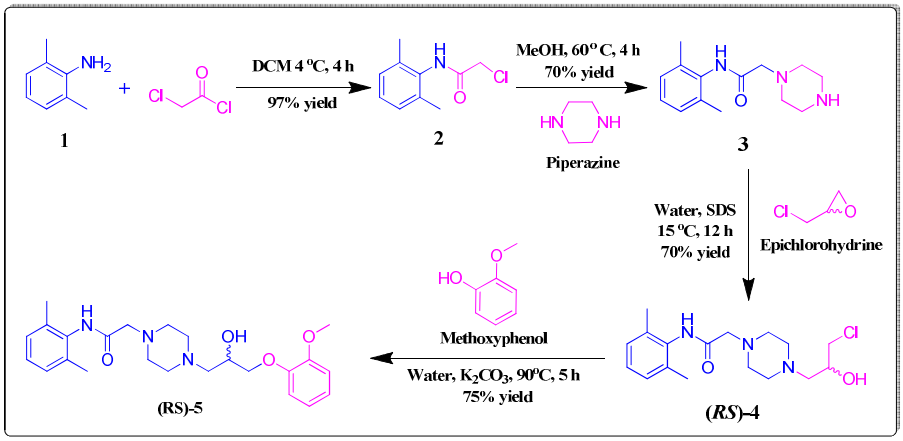
afforded product **4** with 70% yield (Scheme 1). This reaction is a good example of water based chemistry which makes the process green. Likewise, the racemic form of the drug molecule was synthesized by treating compound **4** with methoxyphenol in the presence of K_2CO_3 in water to afford (*RS*)-Ranolazine **5** with 75% yield (Scheme 1). The use of water as a solvent, in the 3rd and 4th step of chemical synthesis makes it attractive in terms of industrial synthesis as it makes the process cost-effective and environment friendly. The current synthetic method is simpler than the previously reported one with comparable yield.[32]



Scheme 1. First step: Synthesis of 2-chloro-N-(2, 6-dimethylphenyl) acetamide 2; Second step: Synthesis of N-(2, 6-dimethylphenyl)-2-piperazin-1-yl)acetamide 3; Third step: Synthesis of (RS)-4 i.e. introduction of chiral center; Fourth step: Synthesis of (RS)-ranolazine 5

2.2 Computational approach for the screening of biocatalysts

Being outstanding biocatalysts, enzymes play dominant role in most of the chemicals reactions.[34] To understand the enzymatic reaction mechanism towards chemical reaction and screening of the suitable biocatalysts, *in silico* docking techniques play an important role.[35] Computational screening of enzymes for biocatalytic reactions can open up a new avenue for green catalysis as it will provide a rational behind the study. Among a variety of docking tools available[36], the Molegro Molecular Viewer 2.5.0 (CLC Bio, Qiagen) program was used for the screening of lipase, as the biocatalyst towards the enantiopure synthesis of ranolazine. Docking of various lipases was carried out with (*RS*)-**5** as the ligand (Zinc 21984084) and molecular docking score and H-bond lengths between the ligand and the various amino acid residues of the enzymes were analyzed. The consequences showed varied docking scores, interaction with the amino acid residues of the typical enzyme and difference in H-bond lengths. Interestingly, the R and S forms of the substrate interacted differently with the same enzyme.[1,37].Only specifically oriented substrate (*R* or *S*) can be interacted significantly with the amino acids present in active site of the enzyme due to distereomeric product formation.

In the present study, the interaction of OH group at the chiral centre of (*RS*)-5 with the amino acid and the distance of the H-bond was considered for the screening of the preeminent apposite enzyme for the kinetic resolution of (*RS*)-5. Out of 11 enzymes screened, the S form of (*RS*)-5 interacted significantly to the amino acid (Glu 67) of *Candida antartica* lipase A CLEA only, with H-bond length of 3.07 Å (Table 1). The Figure 2 represents, -OH group of the ligand (*S*)-**5**, interacted with the carboxyl oxygen of *C. antartica* lipase A showing H-bond but in the opposite direction, here the -OH group of the ligand (*R*)-**5** interacted with the -NH_2 group of Phe 435 in *C. antartica* lipase A CLEA to form H-bond (3.12 Å), which is not an ideal interaction for the transesterification reaction (Figure 3).

The docking score is more negative for (*S*)-**5** [-57.28] than the other enantiomer (*R*)-**5** [-40.67], which clearly indicates better interaction of the lipase with the (*S*)-form and selectivity for acylation.

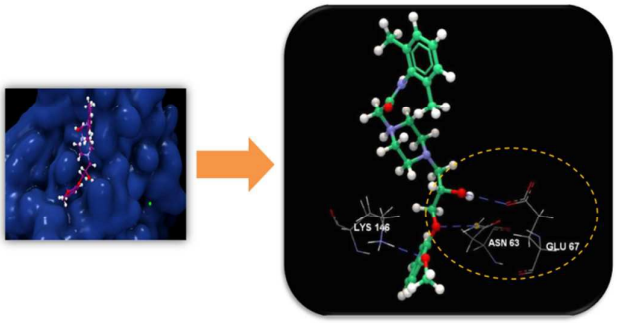


Figure 2. Interaction of (*S*)-5 with CAL-A lipase CLEA. The interaction of amino acid residues of enzyme with the ligand was shown by dotted circle.

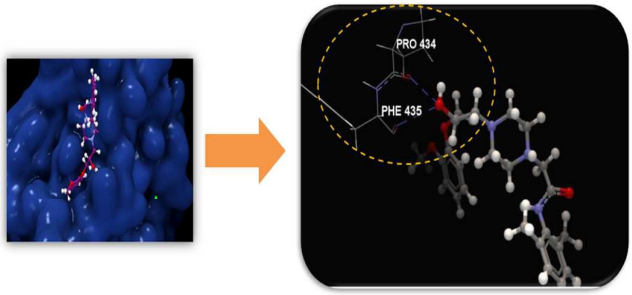


Figure 3. Interaction of (*R*)-5 with CAL-A lipase CLEA. The interaction of amino acid residues of enzyme with the ligand was shown by dotted circle.

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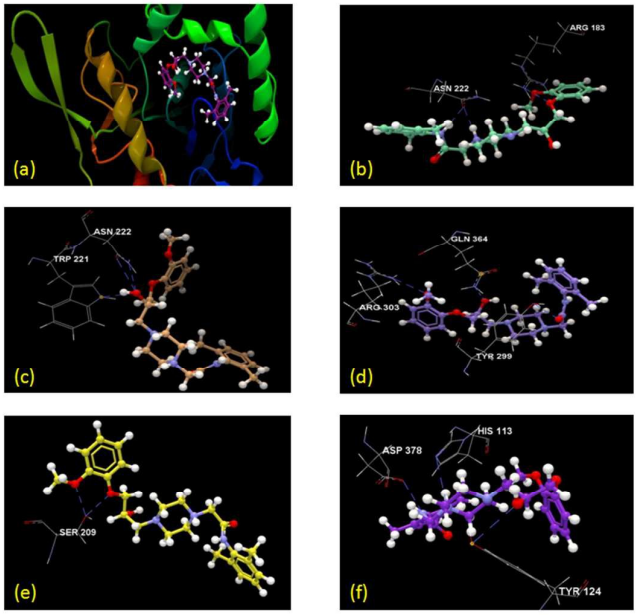


Figure 4. Docking result showing interaction of different lipases with (*S*)-5: (a) 2NW6, (b) 1LPN, (c) 1LPO, (d) 1GZ7, (e) 1CLE, (f) 1UZA

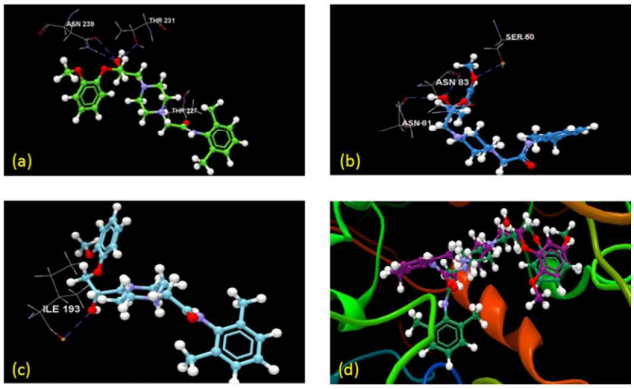


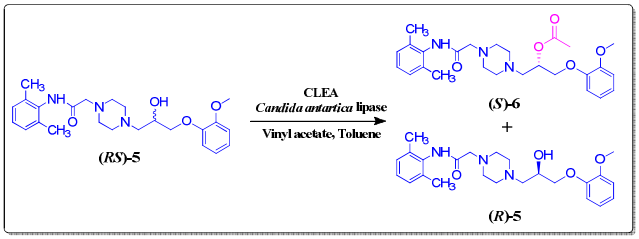
Figure 5. Docking result showing interaction of different lipases with (*S*)-5: (a) 3LIP, (b) 1PCN, (c) 3TGL, (d) 3WNB.

This shows that how the interaction between the chiral substrate (ligand) and the lipase determines the enantioselectivity towards the synthesis of pure enantiomer. Among the other lipases, *C. antartica* lipase acrylic resin and Amano lipase have shown no significant interaction with the substrate (*RS*)-**5** (Table 1) (Figures 4, 5).

Docking with Mucor meihei lipase showed highly positive docking score, indicating poor binding and interaction with the substrate (Table 1). Most of the other lipases showed similar docking score for both the enantiomers, indicating the non-selective binding with the substrate (*RS*)-**5** and resulting in non-selective acylation and low enantiomeric excess.

2.3 Screening of lipases for the kinetic resolution of (*RS*)-5

To support the in-silico data, wet lab screening was performed with various commercial lipases to select the best enzyme on the basis of enantioselectivity, for the kinetic resolution of (*RS*)-**5** (Table 2). Among the different sources, lipase from *Candida cylindricea*, *C. rugosa* CRL L1754, *C. rugosa* CRL 62316, *C. rugosa* CRL 90860, *Aspergillus niger*, immobilized lipase in sol-gel-AK from *Pseudomoas cepacia*, lipase acrylic resin from *C. antartica* lipase A, lipozyme of *Mucor meihei* lipase, cross linked enzyme aggregate of *C. antartica* lipase, Amano-30 lipase, and lipase from porcine pancreas were used for the screening. The biocatalytic reaction was carried out in toluene using vinyl acetate as the acyl donor (Scheme 2).



Scheme 2. Lipase catalyzed kinetic resolution of (RS)-ranolazine (5)

Various lipases were screened and cross-linked enzyme aggregates (CLEA) of *C. antartica* lipase showed 53.55% conversion and enantioselectivity of substrate was very low (31.83%), which is better than the other lipases. To increase the enantiomeric excess of both substrate and product, optimization of various reaction parameters were carried out using CLEA of *C. antartica* lipase as the biocatalyst.

Table 2. Screening of lipase for the kinetic resolution of (*RS*)-ranolazine **5**[a]

Name of lipase	ee_S (%)[b]	ee_P (%)[c]	Conversion (%)[d]	E[e]
C. cylindricea lipase	0.7	2.0	27	1.0
C. antartica lipase A CLEA	*32*	*28*	*54*	*2.3*
Aspergillus niger lipase	3.1	4.0	46	1.1
C. rugosa 62316	1.0	5.5	15	1.1
C. rugosa 90860	0.1	19	31	1.1
C. rugosa L-1754	0.5	5.0	9.0	1.1
Pseudomoas cepacia PCL	5.2	2.3	69	1.0
C. antartica acrylic resin	2.3	4.0	39	1.1
Amano-30 lipase	5.2	2.3	69	1.5
Porcine pancreatic lipase	4.2	3.6	54	1.1
Mucor meihei lipase	0.7	1.0	42	1.1

[a](*RS*)-**5** (10 mM) in toluene (800 µL) was treated with vinyl acetate (200 µL, 2.5 mM) at 30 °C in presence of lipase (15 mg).
[b]Enantiomeric excess of substrate calculated by using formula = (R-S)/(R+S)*100
[c]Enantiomeric excess of product calculated by using formula = (S-R)/(S+R)*100
[d]Conversions were calculated from enantiomeric excess of substrate (ee_S) and product (ee_P) using the formula: Conversion (C) = $ee_S/(ee_S + ee_P)$
[e]E values were calculated using the formula: E = [ln (1 – C (1 + ee_P))]/ [ln (1 –C (1 - ee_P))].

Lipase mediated biocatalytic reactions are highly affected by different physico-chemical parameters such as reaction medium, reaction time, temperature, enzyme and substrate concentration, solvent and type of acyl donor. The enantio-selectivity of the enzyme changes under different reaction conditions. To obtain the ideal reaction condition, effect of various reaction parameters were studied. Post optimization, the most suitable reaction condition was determined i.e. reaction time, 48 h; temperature, 30 °C; enzyme concentration, 30 mg/mL; substrate concentration, 5 mM (Table 3).

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Table 1. *In-Silico* docking study showing the interaction of different lipases with (*S*)/(*R*)-5

Lipase	PDB ID[a]	(*S*)-Ranolazine			(*R*)-Ranolazine		
		Docking score[b]	Steric interaction score	Interacting residues with H-bond distance[c]	Docking score[b]	Steric interaction score	Interacting residues with H-bond distance[c]
Amano-30	2NW6	-54.73	-64.17	No interaction	-67.14	-76.35	No interaction
Candida antartica lipase A CLEA	3GUU	-57.28	-59.02	Asn63-3.034 Å; Glu67-3.071 Å	-40.67	-46.18	Phe435-3.12 Å
Lipase from C. rugosa 62316	1LPN	-36.0	-44.63	Arg183 2.465Å; Asn222-2.113Å	-33.94	-41.61	Gln187-3.05 Å
Lipase from *C. rugosa* 90860	1LPO	-36.19	-35.89	Asn222-3.119 Å; Trp221-2.652 Å	-40.56	-45.6	Asp40-3.170 Å
Lipase *from C. rugosa* L-1754	1GZ7	-51.96	-52.15	Asn351-3.367 Å; Tyr299- 2.659 Å	-47.13	-50.41	Tyr299-3.009 Å; Arg303-3.149 Å
Lipase from *C. cylindricea*	1CLE	-60.34	-65.74	Ser209-2.526 Å	-57.98	-62.22	Ser209-2.515 Å
Lipase from *Aspergillus niger*	1UZA	-47.56	-53.62	His113-3.282 Å; Tyr124-3.385 Å	-49.88	-59.29	No interaction
Lipase *from P. cepacia*	3LIP	-48.69	-50.27	Asn239-3.106 Å; Thr231-2.728 Å	-51.46	-58.23	Leu234-3.106 Å; Ala226-1.730 Å
Porcine pancreatic lipase	1PCN	-52.99	-56.41	Asn81-2.267 Å; Asn83-2.914 Å	-47.81	-58.67	Asn83-2.542Å
Lipase from *Mucor meihei*	3TGL	297.39	280.62	Ile193-3.133 Å	226.7	204.99	No interaction
C. antartica lipase in acrylic resin	3W9B	-63.44	-73.36	No interaction	-66.71	-77.76	No interaction

[a]Protein Data Bank identity number of the lipase
[b]Molecular docking simulation searches through numerous potential binding modes of the ligand (ZINC 21984084) in the pocket denoted as the score, which is calculated by the following formula: Score = $S_{target\text{-}ligand} + S_{ligand}$
[c]H-bond distance of interacting amino acid residue of the enzyme and the ligand (ZINC 21984084)

Table 3. Optimum reaction condition for the kinetic resolution of (*RS*)-ranolazine **5**[a]

Physico-chemical parameters	Optimum value	ee_S (%)[b]	ee_P (%)[c]	Conversion (%)[d]
Reaction time	48 h	73	100	42
Temperature	30 °C	76	100	43
Substrate concentration	5 mM	76	100	43
Enzyme concentration	30 mg/mL	69	100	41

[a]Kinetic resolution of (*RS*)-5 was carried out with *Candida antartica* lipase A CLEA
[b]Enantiomeric excess of substrate calculated by using formula = (R-S)/(R+S)*100
[c]Enantiomeric excess of product calculated by using formula = (S-R)/(S+R)*100
[d]Conversions were calculated from enantiomeric excess of substrate (ee_S) and product (ee_P) using the formula: Conversion (C) = $ee_S/(ee_S + ee_P)$

The effect of solvent on the kinetic resolution of (*RS*)-ranolazine **5** was studied by carrying out the biocatalytic reaction with solvents of different logP values (See supporting information S2). The result showed significant role of reaction medium on the enantioselectivity and conversion of the lipase. Amongst the six different solvents screened, diethyl ether was found to be ideal with conversion of 48% and both high ee_S (90%) and ee_P (98%). Also, the enantiomeric ratio (E) is significantly higher (323) for diethyl ether than the other solvents. Hence, further lipase mediated reactions were carried out using diethyl ether as the solvent (See supporting information S3). The role of acyl donor in the kinetic resolution of (*RS*)-**5** was also studied and vinyl acetate (2.5 mM) was found to be the efficient one with 48% conversion (ee_S 90%, ee_P 99%). The enantiomeric ratio (E) was also higher (588) with vinyl acetate over other acyl donors used.

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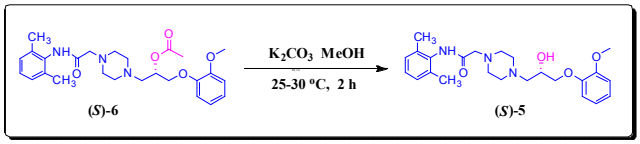
2.4 Synthesis of (S)-ranolazine through deacylation

After successful synthesis of the enantiopure acylated derivative **6**, we focused on the synthesis of our target drug i.e. enantiopure ranolazine. To synthesize (*S*)-ranolazine **5**, the acylated derivative **6** was isolated after the biocatalytic reaction. The acylated derivative, (*S*)-**6** was deacylated in aqueous K_2CO_3 at room temperature for 2 h (Scheme 3) which afforded (*S*)-ranolazine.



Scheme 3. Deacylation of the acylated derivative 6 for the synthesis of (*S*)-Ranolazine **5**

3. Experimental

3.1 General experimental details

3.1.1 Reagents

(*RS*)-glycidol, (*R*)-glycidol, (*S*)-glycidol, 2-methoxyphenol, isopropyl amine and the lipase preparations from *Candida antartica* (CAL) in acrylic resin, *C. rugosa* 62316 (CRL 62316), *C. rugosa* 90860 (CRL 90860), *C. rugosa* L-1754 (CRL L1754), *C. cylindracea* (CCL), *Aspergillus niger* (ANL), cross linked enzyme aggregates (CLEA) of *C. antartica*, *Pseudomonas cepacia* (PCL), immobilized lipase from *Mucor meihei* (MML), and lipase from porcine pancreas (PPL) was purchased from Sigma-Aldrich (USA) and Fluka™. Lipase AY "Amano"30 were purchased from Amano Chem Ltd (USA). The analytical and commercial grade solvents such as hexane, ethyl acetate etc. was procured from various commercial sources. Solvents of HPLC grade such as hexane and 2-propanol were obtained from J. T. Baker.

3.1.2 Analysis

Reactions were analyzed by ^{1}H NMR and ^{13}C NMR spectra, obtained with Bruker DPX 400 (^{1}H 400 MHz and ^{13}C 100 MHz), and chemical shifts were expressed in δ units relative to the tetramethylsilane (TMS) signal as an internal reference in $CDCl_3$. IR spectra (wave number in cm^{-1}) were recorded on Nicolet FT-IR impact 400 instrument. Analytical TLC of all the reactions were carried out on Merck plates. SRL silica gel (60-120 mesh) was used in column chromatography. The enantiomeric excess (*ee*) was determined by HPLC (Shimadzu LC-10AT 'pump, SPD- 10A UV-VIS detector) using a Chiralcel OD-H column (0.46 mm x 250 mm; 5 µm, Daicel, Japan) at 254 nm, with mobile phase, hexane:2-propanol (9:1); flow rate, 1 mL/min and column temperature of 25 °C.

3.2 Synthesis of 2-chloro-*N*-(2,6-dimethylphenyl) acetamide (2)

In the first step, 2, 6-dimethylaniline **1** was treated with chloroacetyl chloride at 4 °C in dichloromethane to obtain 2-chloro-*N*-(2,6-dimethylphenyl)acetamide **2** with 97% practical yield. Recovered product was further analyzed by GC-MS and NMR spectroscopy. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.26 (s, 6H), 4.27 (s, 2H), 7.11-7.19 (m, 3H), 7.28 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 18.32, 42.8, 127.92, 128.39, 132.67, 135.36, 164.38; *m/z*: 197.66 (M= $C_{10}H_{12}ClNO$).

3.3 Synthesis of *N*-(2,6-dimethylphenyl)-2-piperazin-1-yl)-acetamide (3)

For the second step, 2-chloro-*N*-(2,6-dimethylphenyl)-acetamide **2** was reacted with piperazine in methanol at 60 °C to obtain the product **3** with 70% practical yield. Recovered product was further analyzed by GC-MS and NMR spectroscopy. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.25(s, 6H), 2.68-2.69 (d, J= 4 Hz, 4H), 2.97-2.99 (t, 4H), 3.18 (s, 2H), 7.10-7.12 (dd, J= 8 Hz, 3H), 8.67 (s, 1H); *m/z*: 247.95 (M = $C_{14}H_{21}N_3O$).

3.4 Synthesis of (*RS*)-2-(4-(3-chloro-2-hydroxyphenyl)piperazin-1-yl)-*N*-(2,6-dimethylphenyl)acetamide 4

N-(2,6-dimethylphenyl)-2-(piperazin-1-yl)acetamide **3** was treated with (*RS*)-epichlorohydrin in the presence of SDS using water as solvent to obtain (*RS*)-2-(4-(3-chloro-2-hydroxyphenyl)piperazin-1-yl)-*N*-(2,6-dimethylphenyl)acetamide **4** with 70% isolated yield. Recovered product was analyzed by GC-MS and NMR spectroscopy. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.63 (s, 1H), 7.06-7.14 (m, 4H), 4.57 (s, 1H), 3.84-3.88 (dd, J= 16 Hz, 2H), 3.68-3.70 (d, J= 8 Hz, 1H), 3.59-3.61 (dd, J= 8 Hz, 2H), 3.26–3.32 (m, 4H), 2.88 (brs, 4H), 2.82 (s, 1H), 2.26 (brs, 6H); *m/z*: 340.35 (M = $C_{17}H_{26}ClN_3O_2$).

3.5 Synthesis of (*RS*)-ranolazine (5)

To obtain the final drug molecule, (*RS*)-2-(4-(3-chloro-2-hydroxyphenyl)piperazin-1-yl)-*N*-(2,6-dimethylphenyl) acetamide **4** was further treated with methoxy phenol in the presence of K_2CO_3 in water which resulted the synthesis of (*RS*)-Ranolazine **5** with 75% yield. Recovered product was characterized by GC-MS and NMR spectroscopy. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.98–6.96 (m,3H), 6.86–6.77 (m, 4H), 4.06–4.02 (m, 1H), 3.89–3.86 (m, 1H),3.82–3.78 (m, 1H), 3.71 (s, 3H), 2.58 (brs, 8H), 2.55–2.63 (m, 4H) 2.25 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.41, 149.4,148.3, 134.97, 133.61, 128.32, 127.21, 121.97, 120.95, 114.78, 111.9,72.3, 66.0, 61.66, 60.53, 55.8, 53.8, 53.58, 18.67; *m/z*: 428.38 (M= $C_{24}H_{33}N_3O_4$). The product was further characterized by chiral HPLC analysis using chiralcel OD-H column (hexane:2-propanol:: 9:1), t_R = 15 min, t_S = 22.1 min

3.6 In-silico study of screening of lipases for the kinetic resolution of (*RS*)-5

The docking study of (*RS*)-**5** with different lipases was executed in the MVD programme using Docking wizard[38]. In this process, initially eleven different lipases (2NW6, 3GUU, 1LPN, 1LPO, 1GZ7, 1CLE, 1UZA, 3LIP, 1PCN, 3TGL, 3W9B) were imported from PDB (Protein Data Bank) and complexed small molecules were removed. Prior to docking, the docking cavities were recognized using the 'Cavity Prediction' module which utilizes probe (size 1.20 Å) in a grid resolution of 0.80 Å, which expanded van-der-waals molecular surface representation to specify docking site. MolDock scoring function and MolDock Optimizer genetic algorithm was employed to search and score dock poses. MolDock is an extended PLP based docking scoring function with newly added H-bonding and electrostatic energy terms to improve docking accuracy[39]. The best dock poses were retrieved based on the lowest binding energy among multiple poses criteria and after graphical evaluations. The graphical illustrations of protein-ligand interactions were made

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using the Molegro Molecular Viewer (MMV) 2.5.0 programme (CLC Bio, Qiagen Inc.)[40]. Amino acid residues of lipases involved in the interaction with the ligand were determined and respective H-bond lengths were calculated. Also, this interaction with amino acid residues helped to explain the reaction mechanism. The differential interaction of (*R*)/(*S*)-**5** with the lipases were shown through the Molegro Molecular Viewer and it reveals the basis for the enantioselectivity of the biocatalyst.

3.7 Enantioselective transesterification of (*RS*)-5

Commercial lipases from different sources such as *Candida antartica*, *C. rugosa* 90860, *C. rugosa* 62316, *C. rugosa* L-1754, *C. cylindracea*, *Aspergillus niger*, porcine pancreas, AY-Amano 30 and the immobilized lipases like sol-gel-Ak from *Pseudomonas cepacia*, immobilized lipozyme from *Mucor meihei*, lipase acrylic resin from *C. antartica* CLEA, were used for kinetic resolution of (*RS*)-**5**. All the lipases were individually taken into different conical flasks (5 mL). Substrate (**5**) 5 mM in 0.8 mL toluene along with 0.2 mL vinyl acetate (2.5 mM), as acyl donor was added into each flask. The flasks were then capped and kept in an incubator shaker at 37 °C (200 rpm) for 48 h. Reactions were worked up after 48 h and the conversion and enantiomeric excess were analyzed by HPLC.

The effect of different physico-chemical parameters such as organic solvents, reaction time, temperature, enzyme and substrate concentrations, and acyl donors was studied to optimize the reaction condition for achieving the best conversion and highest enantiomeric excess (See supporting information S1, S2, S3).

3.8 Preparative-scale transesterification reaction of (*RS*)-5

The resolution of (*RS*)-**5** was carried out in preparative scale under the optimized condition. The transesterification was performed with 5 mM substrate and *C. antartica* lipase A CLEA at 30 °C using vinyl acetate (2.5 mM) as acyl donor in 50 mL toluene. After 48 h, the reaction mixture was filtered and enzyme preparation was washed with toluene when the transformation was 48.5% with ee_S of 80%. The solvent was evaporated under vacuum and resulting dried residue was subjected to flash chromatography (hexane:ethyl acetate::8:2).

(*S*)-**6**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.7-8.68 (s, 1H), 7.28 (brs, 3H), 7.0-6.91 (dd, 4H), 5.36 (s, 1H), 4.24-4.18 (m, 2H), 3.88-3.87 (d, J= 4 Hz, 3H), 3.24-3.20 (d, J= 16 Hz, 2H), 2.71-2.61 (m, 4H), 2.24 (brs, 6H), 2.09 (s, 2H), 1.59 (brs, 6H); *m/z*: 470.32 (M= $C_{26}H_{35}N_3O_5$). The product was further characterized by chiral HPLC using chiralcel OD-H column (hexane:2-propanol:: 9:1), $t_{S\text{-}6}$ = 13.12 min.

(*R*)-**5**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.98–6.96 (m, 3H), 6.86–6.77 (m, 4H), 4.06–4.02 (m, 1H), 3.89–3.86 (m, 1H),3.82–3.78 (m, 1H), 3.71 (s, 3H), 2.58 (brs, 8H), 2.55–2.63 (m, 4H) 2.25 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.41, 149.4,148.3, 134.97, 133.61, 128.32, 127.21, 121.97, 120.95, 114.78, 111.9,72.3, 66.0, 61.66, 60.53, 55.8, 53.8, 53.58, 18.67; *m/z*: 428.38 (M= $C_{24}H_{33}N_3O_4$); $[\alpha]_D^{20}$= +3.0 (c 4.0, $CHCl_3$). The product was analyzed by HPLC using chiralcel OD-H column (hexane:2-propanol:: 9:1), $t_{R\text{-}5}$ = 15 min.

3.9 Deacylation of (*S*)-6 for the synthesis of (*S*)-5

A solution of K_2CO_3 (0.27 g, 2 mmol) in distilled water (1 mL) was added separately to (*S*)-**6** (1 mmol) in methanol (5 mL) and the reaction mixture was kept under stirring for 2 h at room temperature (30 °C). After completion, the reaction mixture was extracted with EtOAc and water. The combined organic layer was dried over Na_2SO_4 and concentrated under vacuum to

obtain the crude, which was purified by column chromatography (hexane:ethyl acetate:: 8:2) on silica gel (100-200 mesh) to obtain (*S*)-**5** (Scheme 6).

(*S*)-**5**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.98–6.96 (m, 3H), 6.86–6.77 (m, 4H), 4.06–4.02 (m, 1H), 3.89–3.86 (m, 1H), 3.82–3.78 (m, 1H), 3.71 (s, 3H), 2.58 (brs, 8H), 2.55–2.63 (m, 4H) 2.25 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.41, 149.4,148.3, 134.97, 133.61, 128.32, 127.21, 121.97, 120.95, 114.78, 111.9,72.3, 66.0, 61.66, 60.53, 55.8, 53.8, 53.58, 18.67; *m/z*: 428.38 (M= $C_{24}H_{33}N_3O_4$); $[\alpha]_D^{20}$= -3.1 (c 4.0, $CHCl_3$). The product was further characterized by chiral HPLC using chiralcel OD-H column (hexane:2-propanol:: 9:1), $t_{S\text{-}5}$ = 22.1 min.

4. Conclusion

Computational catalysis has become a rationalized approach for *in silico* biocatalysts screening for the synthesis of enantiopure drugs and drug intermediates, as exemplified here using ranolazine as a model drug. The differential interaction of the two enantiomers with lipase, as determined by the docking score and H-bonding pattern, proved to be advantageous to select the suitable enzyme preparation for the kinetic resolution of (*RS*)-Ranolazine. After the *in silico* and wet lab based screening, *Candida antartica* lipase CLEA was found to be the most efficient catalyst for the resolution of (*RS*)-Ranolazine with higher conversion and enantiomeric excess. Under the optimized reaction conditions, 48.5% conversion with 80% enantiomeric excess was achieved which led towards the green synthesis of (*S*)-ranolazine. This combinatorial approach of *in silico* studies and biocatalysis to synthesize enantiopure ranolazine, which avoids the use of toxic metal catalysts, proved to be a game changer in biocatalysis.

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