One-step lipase-catalysed preparation of eslicarbazepine†

M. F. El-Behairyab and E. Sundby*b

The antiepileptic eslicarbazepine (S-licarbazepine) has been prepared in one step from its racemic form RS-licarbazepine via lipase catalysed kinetic resolution. A novel stereoselective simultaneous HPLC separations of RS-licarbazepine (1) and its racemic esters RS-2–5 have been developed on Lux® cellulose-2 column using cyclohexane/ethanol 1/1 v/v as mobile phase. The developed enantioselective HPLC separations have been utilized for monitoring of lipase catalyzed kinetic resolution of RS-licarbazepine (1). Lipase catalysed trans-esterification and hydrolysis reactions have been performed. Four different esters (acetate (2), propionate (3), butyrate (4) and benzoate (5)) have been investigated for both trans-esterification and hydrolysis using ten lipases from versatile origins. The best enantioselectivity was shown by trans-esterification of RS-licarbazepine with vinyl benzoate in MtBE as solvent and lipase from Candida rugosa where the pharmacologically active enantiomer, S-(+)-licarbazepine, has been accomplished [E = 31, ee = 97%, yield 84%, ν301 = +105, c 0.001 g mL−1, CH3OH]. Molecular docking attributed the high enantioselectivity of the transesterification when using Candida rugosa lipase to unfavorable ligand contacts between the S-enantiomer and phenylalanine 296.

1. Introduction

Eslicarbazepine (S-licarbazepine) is the pharmacologically active form of the antiepileptic drugs carbamazepine (CBZ, Tegretol®), oxcarbazepine (OXC, Trileptal®), RS-licarbazepine1 and the key chiral intermediate of eslicarbazepine acetate (Exelief®) and carbamazepine (CBZ) has been utilized as a first-line antiepileptic drug (AED) for focal seizures. However, the toxic 10,11-epoxide metabolite of CBZ (Fig. 1) led to the introduction of the second-generation oxcarbazepine to decrease side effects.2 In humans, oxcarbazepine was found to be stereoselectively reduced to licarbazepine (8 : 2 : S : R)3.

Several approaches have been reported for production of eslicarbazepine. These approaches comprises diastereomeric crystallization by p-Me dibenzoyl tartaric acid,4 diacetyl tartrate,5 S-ibuprofen,5–7 naproxen8 or acetyl (R)-mandelic acid.9 Also, asymmetric reduction of oxcarbazepine has been reported,8,10–17 These methods have been very well summarized by N. K. Modukuru et al. who has also developed a biocatalytic reduction of oxcarbazepine using an evolved ketoreductase.18 Further biocatalysed reduction has been established by Zhi-Min Ou et al. where Saccharomyces cerevisiae has been used.19 Finally, Ravinder et al. has synthesized eslicarbazepine acetate by employing chemical enantioselective reduction and carboxamidation reaction.20

In a closely related work, eslicarbazepine has been prepared starting from racemic licarbazepine via four steps (Fig. 2). The later method includes chemical esterification, enzymatic hydrolysis of methoxyacetate ester, removal of undesired alcohol by hemisuccinate formation and sodium hydroxide hydrolysis of isolated ester.21

In the present work, one-step, practical, and scalable enzyme-mediated process for the production of (S)-licarbazepine that provides important benefits over previously described whole cell processes and chemo-catalytic processes has been developed.

†Electronic supplementary information (ESI) available: IR, MS and NMR spectra of selected compounds, chiral HPLC separation of compound 1–5 (pure compounds and time resolved transesterification) and 2D ligand–protein interaction maps after docking of compound 2–5. See DOI: 10.1039/c6ra23915c

Fig. 1 Metabolism of carbamazepine, oxcarbazepine, and eslicarbazepine acetate in humans.
2. Experimental

2.1. Materials and reagents

HPLC grade n-hexane, c-hexane, methanol, ethanol, acetonitrile and 2-propanol were from Sigma chemicals (St. Louis, MO, USA). Lipases (Candida rugosa (L1754), lipase A, Candida antarctica, (12117), lipase from Pseudomonas stutzeri CLEA (CLEA117-ST), lipase from porcine pancreas (L3126), lipase B Candida antarctica immobilized on Immobead 150, recombinant from Aspergillus oryzae (54326), lipase from Aspergillus niger (62301), lipase, immobilized in Sol-Gel-AK from Pseudomonas cepacia (62279), lipase, Thermomyces lanuginosus, CLEA (07676)) were purchased from Sigma chemicals. Lipozyme RM IM and Novozyme 435 were from Novozyme®. Oc-xcarbazepine and eslicarbazepine acetate were provided by local pharmaceutical companies.

2.2. Instrumentation

1H NMR (400 MHz) and 13C NMR (100 MHz) spectra were performed on Jeol ECA spectrometer using TMS as internal standard and chemical shift values were recorded in ppm on δ scale. The 1H NMR data were represented as follows: chemical shifts, multiplicity (s. singlet, d. doublet, dd. doublet of doublet, t. triplet, m. multiplet), number of protons. The 13C NMR data were represented as chemical shift values were recorded in ppm on scale. Melting points were determined using Electrothermal Capillary melting point apparatus and are uncorrected. The HPLC unit was Agilent 1100 pharmaceutical companies.

2.3. Synthetic procedures

2.3.1. Synthesis of RS-lcarbazepine (RS-1). NaBH₄ (2.25 g, 0.06 mol) was added portion-wise to an ice cooled, stirred solution of oxcarbazepine (4 g, 0.015 mol) in methanol (50 mL). The mixture was stirred overnight at ambient temperature followed by addition of water and evaporation under vacuum. The residue was dissolved in ethyl acetate/water (300/100 mL) and the organic layer was separated, dried (Na₂SO₄) and evaporated under reduced pressure to afford 3.2 g (80%) of RS-lcarbazepine as white powder mp 198 °C (literature 195–196 °C (ref. 22)). IR cm⁻¹: 3491 (NH), 3368 (OH), 1677 (amidic C=O). Ms: C₁₁H₁₄N₁O₂ calc. 254.28, found (M⁺) 254.19.

2.3.2. General procedures for synthesis of RS-lcarbazepine esters. Appropriate acid anhydride (0.012 mol) were added to a stirred solution of RS-lcarbazepine (1) (0.5 g, 0.002 mol) in pyridine. The reaction mixture was refluxed while stirred overnight then poured over aqueous HCl (50 mL, 10%) and extracted with ethyl acetate (2 × 50 mL). The organic layer was separated, dried (Na₂SO₄) and evaporated under vacuum to afford crude esters. The crude esters were recrystallized from ethanol to afford pure esters.

2.3.2.1. (RS)-10-Acetoxy-10,11-dihydro-5H-dibenz[b,f]azepine-5-carboxamide (2). Yield 0.5 g (86%), white solid, mp 164–166 °C (literature²³ 159 °C). IR cm⁻¹: 3473, 3344 (NH₂), 1723 (ester C=O), 1652 (amidic C=O). Ms: C₁₁H₁₄N₁O₄ calc. 296.12, found (M+) 296.10. 1H-NMR (400 MHz, CDCl₃) δ 7.46–7.26 (m, 8H), 4.83 (s, 2H, NH₂), 3.59 (s, 1H), 3.20–3.08 (m, 1H), 2.10 (s, 3H), 1.66 (s, 1H).

2.3.2.2. (RS)-10,11-Dihydro-10-propionyloxy-5H-dibenz[b,f]azepine-5-carboxamide (3). Yield 0.52 g (85%), white solid mp 176–178 °C (literature²³ 141–143 °C), IR cm⁻¹: 3473, 3362 (NH₂), 1723 (ester C=O), 1650 (amidic C=O). Ms: C₁₃H₁₄N₁O₄ calc. 310.13, found (M+) 310.17. 1H NMR (400 MHz, CDCl₃) δ 7.48–7.18 (m, 8H), 4.81 (s, 2H, NH₂), 3.60 (d, J = 13.4 Hz, 1H), 3.27–3.01 (m, 1H), 2.34 (s, 2H), 1.76–1.65 (m, 2H), 1.63 (s, 1H), 0.99 (t, J = 7.48 Hz, 3H).

2.3.2.3. (RS)-10-Butyroxy-10,11-dihydro-5H-dibenz[b,f]azepine-5-carboxamide (4). Yield 0.25 g (40%), white solid mp 136–137 °C (literature²³ 167–169 °C), 1H NMR (400 MHz, CDCl₃) δ 7.54–7.16 (m, 8H), 4.76 (s, 2H, NH₂), 3.63 (d, J = 14.0 Hz, 1H), 3.19–3.06 (m, 1H), 2.37 (m, 2H), 1.70 (m, 2H), 1.63 (s, 1H), 0.99 (t, J = 7.3 Hz, 3H).

2.3.2.4. (RS)-10-Benzoyloxy-10,11-dihydro-5H-dibenz[b,f]azepine-5-carboxamide (5). Yield 0.35 g (50%), white solid mp 182–183 °C (literature²³ 179–180 °C). 1H NMR (400 MHz, CDCl₃) δ 8.23–8.03 (m, 1H), 7.98 (d, J = 6.7 Hz, 1H), 7.63–7.13 (m, 11H), 4.96 (d, J = 30.0 Hz, 2H, NH₂), 3.74 (dd, J = 18.4, 11.2 Hz, 1H), 3.41–3.13 (m, 1H), 1.37–1.20 (m, 1H).

2.3.3. General procedure for lipase catalyzed transesterification. RS-Lcarbazepine (RS-1) (10 mg, 0.04 mmol) was dissolved in MeBE (5 mL) in a 12 mL glass vial followed by addition of acyldonor (0.12 mmol, 3 eq.) and lipase (40 mg). The mixture was heated to 40 °C, stirred at 300 rpm. A sample of 50 µL was collected daily, diluted with ethanol to 300 µL then 20 µL was injected on HPLC system equipped with Lux cellulose-2 column.

2.3.4. General procedure for lipase catalyzed hydrolysis. RS-Lcarbazepine ester (RS-2) (0.04 mmol) was dispersed in phosphate buffer pH 7.0 (5 mL) in a 12 mL glass vial followed by addition of the lipase (40 mg). The mixture was heated to 40 °C, stirred at 300 rpm. A sample of 50 µL was collected daily, extracted with MeBE, diluted with ethanol and 20 µL was injected on the Lux 3 µ cellulose-2 column.
2.3.5. Lipase catalyzed synthesis of S-licarbazepine. 500 mg of RS-1 was dissolved in 250 mL MfBE then lipase (500 mg) and vinyl benzoate (544 μL, 582 mg, 2 equivalents) were added. The mixture kept stirring at 40 °C while monitored by chiral HPLC using Lux 3 μ cellulose-2 and cyclohexane/ethanol as mobile phase. Once the R-1 was fully converted to R-5 the reaction was stopped by filtering off the enzyme and the S-1 was separated from R-5 and S-5 by silica column using CHCl₃/ethyl acetate as solvent to afford 220 mg (84%) of S-licarbazepine (S-1) [E = 31, ee = 97%, α₁₀⁵ = +105, c 0.001 g mL⁻¹, CH₃OH] as white solid.

2.4. Molecular modelling

The X-ray crystal structures of the protein 1LPM (Candida rugosa lipase) and 1LBT (Candida antarctica lipase B) were prepared using the protein preparation wizard, which is part of the Maestro software package (Maestro, v8.5; Schrödinger, LLC, New York, NY, USA) using the OPLS-3 force field. The resulting protein structures were used in the following docking study. Ligands were drawn using the Maestro 2D Sketcher tool and were prepared using LigPrep2.2 (LigPrep, v2.2; Schrödinger, LLC). For the computational investigation of the lipase-substrate structures, the energy minimized structures of 1LPM and 1LBT and ligands were subsequently docked using Glide, and the resulting docked poses were analysed using the Glide pose viewer tool from Schrödinger.

3. Results and discussion

3.1. Chemistry

RS-Licarbazepine (1) and esters 2–5 have been synthesized as depicted in Scheme 1.

A modified method of that developed by Hirpara et al., has been used where no pH adjustment is needed and only methanol has been used as solvent. The afforded alcohol, RS-1, has been confirmed by IR where new and broad OH band has been observed at 3368 cm⁻¹ in addition to the NH band at 3491. Also, mass spectroscopy has shown molecular ion peak (M⁺) 254.19, M – 18 signal (dehydrated product) (M⁺) 236.20 and the de-carboxamide product at 193.13 (see ESI Fig. S1 and S2†). Further reaction of RS-1 with the appropriate acid anhydride in pyridine afforded the corresponding esters RS-2–5. Esters were purified by crystallization form ethanol and confirmed by different spectroscopic techniques (see experimental and ESI Fig. S3–10†).

Scheme 1 Preparation of RS-licarbazepine (1) and its ester RS-2–5.

3.2. Enantioselective analysis

Enantioselective chromatography has been documented as modern sensitive and most widely used method for the estimation of kinetic resolution reaction outcomes (enantiomeric excess (ee) and enantiomeric ratio or enantioselectivity (E)). The reliance on chromatography has been driven by developments in chiral stationary phases (CSPs) that revealed reliable, robust, and efficient resolution in a timely fashion. Among all used CSPs in HPLC, polysaccharide based CSPs has been recognized as the most powerful and successful for a wide range of chiral molecules. Thus, both columns have been investigated to achieve simultaneous baseline separation of both reaction substrates and the expected products.

Different mobile phases (ethanol 100%, methanol 100%, acetonitrile 100% and n-hexane or cyclohexane/ethanol 1/1 v/v) have been considered. For the amylose-2 column, absolute methanol or acetonitrile showed no chiral recognition abilities for the compounds (1–5). While absolute ethanol and n-hexane/ethanol mixture was able to resolve the acetate and propionate esters, licarbazepine was not resolved (Fig. S11†).

Using the Lux cellulose-2 column, methanol 100% was able to give baseline separation of RS-licarbazepine (1) but only partial separation of the acetate and propionate esters (Fig. S12†). On the other side, acetonitrile, n-hexane/ethanol 1/1 v/v and cyclohexane/ethanol 1/1 v/v as mobile phases gave baseline resolution of RS-1 and all esters (RS-2–5) (Fig. S13†). Based on the runtime, peak sharpness and resolution, cyclohexane/ethanol 1/1 v/v has been selected for monitoring of lipase-catalysed reactions (Fig. 3 and Table 1).

3.3. Biocatalysed kinetic resolution of RS-licarbazepine (1)

Recently, biotransformation became a well-established substitute to conventional chemical methods. As unique and ubiquitous enzyme, lipases are the most widely used class of biocatalysts. This includes their applications in organic synthesis and kinetic resolution of racemic compounds.
Table 1  Separation parameters of RS-licarbazepine (1) and its racemic esters (2–5) using the Lux cellulose-2 column and cyclohexane/ethanol 1/1 v/v

<table>
<thead>
<tr>
<th>Compound</th>
<th>$t_1$</th>
<th>$t_2$</th>
<th>$R_s$</th>
<th>$\alpha$</th>
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<tr>
<td>1</td>
<td>8.4</td>
<td>14.58</td>
<td>9.46</td>
<td>1.72</td>
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<tr>
<td>2</td>
<td>11.46</td>
<td>13.30</td>
<td>2.96</td>
<td>1.16</td>
</tr>
<tr>
<td>3</td>
<td>10.19</td>
<td>12.08</td>
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</tr>
<tr>
<td>4</td>
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<td>5</td>
<td>10.61</td>
<td>11.63</td>
<td>2.26</td>
<td>1.13</td>
</tr>
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</table>

At the inception, a series of three lipases has been selected for screening of different acyl donor for trans-esterification of RS-licarbazepine. Thus, lipases from Candida antarctica A and B and lipase from Candida rugosa have been used for trans-esterification of RS-licarbazepine with vinyl acetate, propionate, butyrate and benzoate. Methyl tert-butyl ether (MβBE) was used as solvent due to week solubility of RS-licarbazepine in hydrophobic solvents like hexane (Table 2) (Scheme 2).

Surprisingly, lipase B from Candida antarctica was completely inactive while lipase A was non-selective for all used acyl donors. Lipase from Candida rugosa displayed the best activity and selectivity (Table 2). Vinyl propionate and butyrate as acyl donors exhibited mediocre enantioselectivity, but the best enantioselectivity was demonstrated when using vinyl benzoate as acyl donor (Table 2 and Fig. S14–S17†).

Further lipases have been screened using vinyl benzoate as acyl donor in order to explore additional enantioselective transesterifications. However, lipase from Candida rugosa kept showing superior performance with respect to activity and selectivity (Table 3).

Since lipases are catalysing reactions in both aqueous and organic phases. It was worthy to check the ingenuity of lipase to hydrolyse RS-licarbazepine esters (2–5) selectively. Consequently, lipase from Candida rugosa was used for hydrolysis of RS-licarbazepine esters (2–5) in aqueous medium (phosphate buffer pH 7.0). Lipases are expected to show higher activity in aqueous solution as native media than in organic solvent. In fact, high activity has been observed however the enantioselectivity was insufficient for further investigations (Table 4).

Based on the results of the above investigations, lipase from Candida rugosa and vinyl benzoate was selected for scaling up the reaction. Thus, 500 mg of RS-1 was dissolved in 250 mL MβBE, then 500 mg of lipase and vinyl benzoate (544 µL, 582 mg, 2 equivalents) were added. The mixture was kept stirring at 40 °C while monitored by chiral HPLC using Lux 3 µ cellulose-2 and cyclohexane/ethanol as mobile phase (Scheme 3 and Fig. 4). Once the R-1 was fully converted to $S$-1 the reaction was stopped by filtering off the enzyme and $S$-1 was separated from R-5 and S-5 by silica column chromatography using CHCl₃/ethyl acetate (1 : 1) as eluent to afford 220 mg (84%) of S-licarbazepine (5) [E = 31, ee = 97%, $\alpha_d^{20} = +105$, c 0.001 g mL⁻¹, CH₃OH] as white solid. The enantiomeric purity of the product has been confirmed with enantioselective chromatography as seen in Fig. 5.

Table 2  Lipases catalysed trans-esterification of RS-licarbazepine

<table>
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<tr>
<th>Lipase</th>
<th>Acyl donor</th>
<th>$T$ (d)</th>
<th>ee&lt;sub&gt;s&lt;/sub&gt;</th>
<th>ee&lt;sub&gt;p&lt;/sub&gt;</th>
<th>Conv.</th>
<th>E</th>
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</thead>
<tbody>
<tr>
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<td>5</td>
<td>3.1</td>
<td>3.2</td>
<td>49.4</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Butyrate</td>
<td>6</td>
<td>26.7</td>
<td>18.7</td>
<td>58.7</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Propionate</td>
<td>6</td>
<td>38.3</td>
<td>29.8</td>
<td>56.2</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Acetate</td>
<td>5</td>
<td>49.8</td>
<td>35.4</td>
<td>58.4</td>
<td>3.3</td>
</tr>
<tr>
<td>C. rug.</td>
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<td>96.8</td>
<td>77.1</td>
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</tr>
<tr>
<td></td>
<td>Butyrate</td>
<td>6</td>
<td>42.3</td>
<td>72.6</td>
<td>36.8</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>Propionate</td>
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<td>34.4</td>
<td>62.0</td>
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<tr>
<td></td>
<td>Acetate</td>
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<td>30.7</td>
<td>28.1</td>
<td>52.1</td>
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</tbody>
</table>

Table 3  Screening of lipases for trans-esterification of RS-licarbazepine (1) using vinyl benzoate in MβBE

<table>
<thead>
<tr>
<th>Lipase</th>
<th>Time (d)</th>
<th>ee&lt;sub&gt;s&lt;/sub&gt;</th>
<th>ee&lt;sub&gt;p&lt;/sub&gt;</th>
<th>Conv.</th>
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<td>74.6</td>
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<td>Lipozyme RM</td>
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<td>P. stutzeri</td>
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<tr>
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<td>7</td>
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<td>—</td>
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<td>—</td>
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<tr>
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<td>77.1</td>
<td>55.6</td>
<td>31.3</td>
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<sup>a</sup> Lipase from porcine pancreas. <sup>b</sup> No reaction.
3.4. Molecular modeling and enantiopreference

Lipase B from *Candida antartica* is probably the most utilized hydrolytic enzyme for kinetic resolutions and contains a so-called ‘stereoselective pocket’ which gives the enzyme high substrate selectivity toward various sec-alcohols. However, this pocket is only large enough to accommodate an ethyl or smaller substituent under standard conditions, which explains the unreactivity towards vinyl benzoate and licarbazepine (Table 3). When attempting to dock substrates 2–5 as benzoate esters on this lipase (PDB entry 1PLS) using Glide, this was also evident, as all poses did not penetrate very deep

<table>
<thead>
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<th>Substrate</th>
<th>Docking score (kcal mol(^{-1}))</th>
<th>(E)</th>
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<tr>
<td>R-5</td>
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<tr>
<td>S-5</td>
<td>-6.518</td>
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<tr>
<td>R-4</td>
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<td>R-3</td>
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<td>S-4</td>
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<tr>
<td>R-2</td>
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<tr>
<td>S-3</td>
<td>-5.070</td>
<td>5.94</td>
</tr>
</tbody>
</table>

Table 5 Docking scores of the best poses of both enantiomers of substrates 2–5

Fig. 4 HPLC traces of large scale transesterification of RS-licarbazepine (I) using vinyl benzoate lipase from *Candida rugosa* in MiBE.

Fig. 5 HPLC traces of enantiopure S-licarbazepine (S-1) after column chromatography (silica, CHCl₃/ethyl acetate) using Lux cellulose-2 column and cyclohexane/ethanol 1/1 v/v.

Fig. 6 Best docking poses for substrate S-5 (top) and R-5 (bottom) on *Candida rugosa* lipase (PDB entry 1LPM). The ligands are shown in green and unfavorable contacts are shown in yellow dotted lines.
into the stereoselectivity pocket and all poses also gave high energy docking scores.

In order to explain the enantioselective hydrolytic reactions using the lipase from Candida rugosa, we performed similar docking experiments with substrates 2–5 (PDB entry 1LPM), Table 5. 2D interaction plot for the best docking poses for all enantiomers are shown in S18 (ESI†). The results show good agreement in difference in docking scores between the enantiomers and the corresponding enantioselectivity ratio.

As can be seen from Table 5, the docking results supports the enantioselectivity observed in the hydrolytic reactions. For all enantiomeric pairs, the docking score for the R enantiomer surpasses the scores for the S-enantiomer. The enantioselectivity of Candida rugosa lipase is claimed to be dependent on selective interaction with the phenylalanine residue at the mouth of the hydrophobic channel close to the active site at position 296. This is also evident in the best docking poses of substrate 5. Phenylalanine 296 shows unfavorable ligand contacts towards both the phenyl ring of the benzoate ester and towards a hydrogen on the central 7-membered ring on the S-5 enantiomer, while the R-5 enantiomer do not show similar unfavorable contacts (Fig. 6).

4. Conclusions

In this work, a new, eco-friendly, one step biocatalysed preparation of eslicarbazepine has been performed. The new access to enantiopure eslicarbazepine offers feasible, reproducible and economic technique in comparison to the tedious and time consuming diastereomeric crystallization methods or stereoselective reduction using ketoreductase which is expensive and limited to aqueous media. Using vinyl benzoate as acyl donor led to a one-step route instead of the multistep process described by Husain, M. and D. Datta. Being a biotransformation, the current method provides green access to eslicarbazepine if compared to chemical stereoselective reduction. Further, enantioselective HPLC separation of R5-larcarbazepine (1) and its racemic esters RS-2-5 has been investigated where Lux® cellulose-2 column and a mobile phase consisting of c-hexane/ethanol 1/1 v/v provoked the anticipated simultaneous baseline separations of esters and the free alcohol. Transesterification of RS-larcarbazepine with vinyl benzoate in MeBE by lipase from Candida rugosa afforded S(+)larcarbazepine in good yield and high ee. [E = 31, ee = 97%, yield 84%, aD = +105, c 0.001 g mL−1, CH3OH]. Docking experiments attributed the enantioselectivity to unfavorable ligand contacts between the S-enantiomer of 1 and phenylalanine 296 in the Candida rugosa lipase.

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Notes and references

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