Published on 10 October 2016. Downloaded by Norwegian University of Science and Technology on 4/23/2020 8:11:41 AM.

RSC Advances

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2016, **6**, 98730

One-step lipase-catalysed preparation of eslicarbazepine†

M. F. El-Behairy[ab] 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 *Mt*BE as solvent and lipase from *Candida rugosa* where the pharmacologically active enantiomer, *S*-(+)-licarbazepine, has been accomplished [$E = 31$, ee = 97%, yield 84%, $\alpha_D^{20} = +105$, c 0.001 g mL^{-1}, CH_3OH]. 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.

Received 26th September 2016
Accepted 7th October 2016

DOI: 10.1039/c6ra23915c

www.rsc.org/advances

1. Introduction

Eslicarbazepine (*S*-licarbazepine) is the pharmacologically active form of the antiepileptic drugs carbamazepine (CBZ, Tegretol®), oxcarbazepine (OXC, Trileptal®), *RS*-licarbazepine[1] 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,[6,7] naproxen[8] 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.

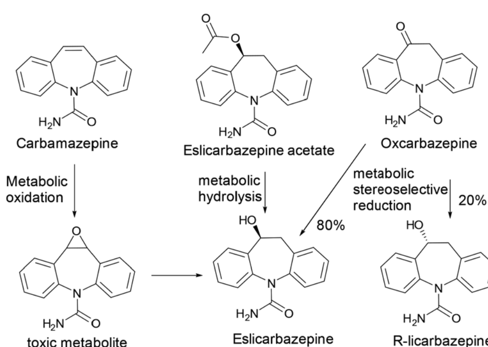


Fig. 1 Metabolism of carbamazepine, oxcarbazepine, and eslicarbazepine acetate in humans.

[a]*Medicinal and Pharmaceutical Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre (ID 60014618), Dokki, Giza, Egypt*

[b]*Norwegian University of Science and Technology, Institute of Chemistry and Material Technology, Faculty of Technology, NTNU, 7491 Trondheim, Norway. E-mail: eirik.sundby@ntnu.no*

† 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

 This journal is © The Royal Society of Chemistry 2016

Published on 10 October 2016. Downloaded by Norwegian University of Science and Technology on 4/23/2020 8:11:41 AM.

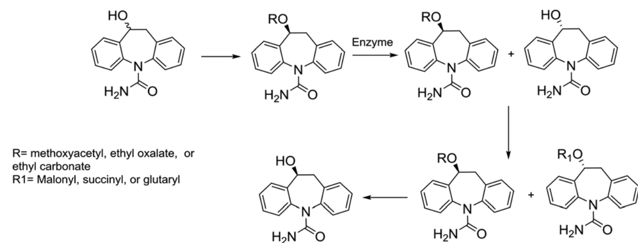


Fig. 2 Preparation of eslicarbazepine *via* 4 steps process as described by M. Husain and D. Datta.[21]

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 lanuginosa*, CLEA (07676)) were purchased from Sigma chemicals. Lipozyme RM IM and Novozyme 435 were from Novozyme®. Oxcarbazepine and eslicarbazepine acetate were provided by local pharmaceutical companies.

2.2. Instrumentation

^{1}H NMR (400 MHz) and ^{13}C 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 ^{1}H 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 ^{13}C NMR data were represented as chemical shifts. Melting points were determined using Electrothermal Capillary melting point apparatus and are uncorrected. The HPLC unit was Agilent 1100 series apparatus equipped with a quaternary pump, a vacuum degasser, autosampler, column compartment, a diode array UV-detector. The signal was acquired and processed by HP Chemstation software. The columns used were Lux 3μ amylose-2 (amylose tris(5-chloro-2-methylphenyl carbamate)) and Lux 3μ cellulose-2 (cellulose tris(3-chloro-4-methyl phenylcarbamate)) (Phenomenex, Le Pecq, France). The dimensions of both columns are 250 mm × 4.6 mm, 3 μm. The flow rate was 1 mL min^{-1}. All the samples were measured at wavelength 254 nm at 25 °C.

2.3. Synthetic procedures

2.3.1. Synthesis of *RS*-licarbazepine (*RS*-1). $NaBH_4$ (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 (Na_2SO_4) and evaporated under reduced pressure to afford 3.2 g (80%) of *RS*-licarbazepine as white powder mp 198 °C (literature 195–196 °C (ref. 22)). IR cm^{-1}: 3491 (NH), 3368 (OH), 1677 (amidic C=O). Ms: $C_{15}H_{14}N_2O_2$ calc. 254.28, found (M$^+$) 254.19.

2.3.2. General procedures for synthesis of *RS*-licarbazepine esters. Appropriate acid anhydride (0.012 mol) were added to a stirred solution of *RS*-licarbazepine (**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 (Na_2SO_4) 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 (literature[23] 159 °C). IR cm^{-1}: 3473, 3344 (NH_2), 1723 (ester C=O), 1652 (amidic C=O). Ms: $C_{17}H_{16}N_2O_3$ calc. 296.12, found (M$^+$) 296.10. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.46–7.26 (m, 8H), 4.83 (s, 2H, NH_2), 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 (literature[23] 141–143 °C), IR cm^{-1}: 3473, 3362 (NH_2), 1723 (ester C=O), 1650 (amidic C=O). Ms: $C_{18}H_{18}N_2O_3$ calc. 310.13, found (M$^+$) 310.17. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48–7.18 (m, 8H), 4.81 (s, 2H, NH_2), 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 (literature[23] 167–169 °C), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.16 (m, 8H), 4.76 (s, 2H, NH_2), 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 (literature[23] 179–180 °C). ^{1}H NMR (400 MHz, $CDCl_3$) 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, NH_2), 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 trans-esterification. *RS*-Licarbazepine ((*RS*)-**1**) (10 mg, 0.04 mmol) was dissolved in M*t*BE (5 mL) in a 12 mL glass vial followed by addition of acyl donor (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*-Licarbazepine ester ((*RS*)-**2–5**) (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 M*t*BE, diluted with ethanol and 20 μL was injected on the Lux 3 μ cellulose-2 column.

Published on 10 October 2016. Downloaded by Norwegian University of Science and Technology on 4/23/2020 8:11:41 AM.

2.3.5. Lipase catalyzed synthesis of *S*-licarbazepine. 500 mg of *RS*-**1** was dissolved in 250 mL M*t*BE 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 $CHCl_3$/ethyl acetate as solvent to afford 220 mg (84%) of *S*-licarbazepine (*S*-1) [$E = 31$, ee = 97%, $\alpha_D^{20} = +105$, c 0.001 g mL^{-1}, CH_3OH] as white solid.

2.4. Molecular modelling

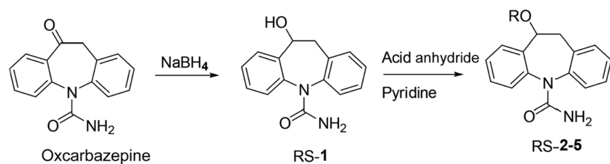
The X-ray crystal structures of the protein 1LPM (*Candida rugosa* lipase) and 1LBT (*Candida antarctica* lipase B) were prepared using the protein preparation wizard, which is part of the Maestro software package (Maestro, v8.5; Schrödinger, LLC, New York, NY, USA) using the OPLS-3 force field. The 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,[24] 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.*,[4] 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 decarboxamide 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)).[25] 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.[26–28] Lux® 3 μ, amylose-2 and cellulose-2 have been recently launched and proved potency in enantioselective resolution of racemic compounds.[29–31] 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.[32] As unique and ubiquitous enzyme, lipases are the most widely used class of biocatalysts.[33] This includes their applications in organic synthesis and kinetic resolution of racemic compounds.[34]

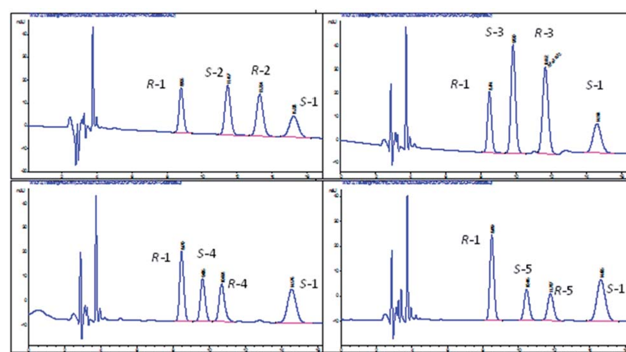


Fig. 3 HPLC chromatograms showing simultaneous baseline separation of *RS*-licarbazepine (**1**) and esters (**2–5**) using Lux cellulose-2 column and cyclohexane/ethanol 1/1 v/v.

 This journal is © The Royal Society of Chemistry 2016

Published on 10 October 2016. Downloaded by Norwegian University of Science and Technology on 4/23/2020 8:11:41 AM.

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

Compound	t_1	t_2	R_s	α
1	8.4	14.58	9.46	1.72
2	11.46	13.30	2.96	1.16
3	10.19	12.08	3.33	1.19
4	9.79	10.84	1.98	1.11
5	10.61	11.63	2.26	1.13

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 (M*t*BE) was used as solvent due to week solubility of *RS*-licarbazepine in hydrophobic solvents like hexane (Table 2) (Scheme 2).

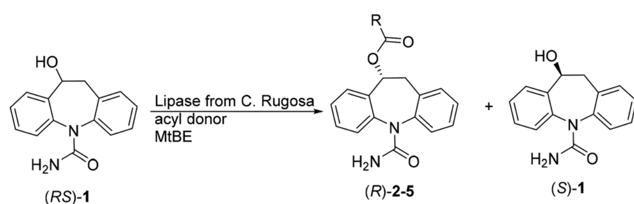
Surprisingly, lipase B from *Candida antarctica* was completely inactive while lipase A was non-selective for all used acyl donors. Lipase from *Candida rugosa* displayed the best activity and selectivity (Table 2). Vinyl propionate and butyrate as acyldonors exhibited mediocre enantioselectivity, but 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

Table 2 Lipases catalysed trans-esterification of *RS*-licarbazepine

Lipase	Acyl donor	T (d)	ee$_s$	ee$_p$	Conv.	E
C. ant.-A	Benzoate	5	3.1	3.2	49.4	1.1
	Butyrate	6	26.7	18.7	58.7	1.8
	Propionate	6	38.3	29.8	56.2	2.6
	Acetate	5	49.8	35.4	58.4	3.3
C. rug.	Benzoate	5	96.8	77.1	55.6	31.3
	Butyrate	6	42.3	72.6	36.8	9.5
	Propionate	6	34.4	62.0	35.6	5.9
	Acetate	5	30.7	28.1	52.1	2.3



Scheme 2 Transesterification of *RS*-licarbazepine (**1**).

Table 3 Screening of lipases for trans-esterification of *RS*-licarbazepine (**1**) using vinyl benzoate in M*t*BE

Lipase from	Time (d)	ee$_s$	ee$_p$	Conv.	E
T. lanuginosa	7	3.4	11.8	22.6	1.3
Amano 20	7	22.5	74.6	23.2	8.5
Lipozyme RM	5	2.4	40.7	5.6	2.4
P. stutzeri	7	—[b]	—	—	—
PPL[a]	7	—	—	—	—
C. antarctica-B	7	—	—	—	—
P. cepacia	7	—	—	—	—
A. niger	7	—	—	—	—
C. antarctica-A	5	3.1	3.2	49.4	1.1
C. rugosa	5	96.8	77.1	55.6	31.3

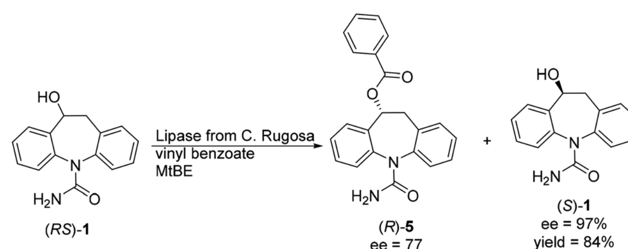
[a] Lipase from porcine pancreas. [b] No reaction.

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 M*t*BE, 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 *R*-**5** 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 $CHCl_3$/ethyl acetate (1 : 1) as eluent to afford 220 mg (84%) of *S*-licarbazepine (*S*-**1**) [$E = 31$, ee = 97%, $\alpha_D^{20} = +105$, c 0.001 g mL^{-1}, CH_3OH] as white solid. The enantiomeric purity of the product has been confirmed with enantioselective chromatography as seen in Fig. 5.

Table 4 Screening of lipases for hydrolysis of *RS*-licarbazepine esters (**2–5**)

Ester	Time (h)	ee$_s$	ee$_p$	Conv.	E
2	48	51.18	38.82	56.86	3.64
3	48	19.51	4.28	81.98	1.26
4	48	48	1.11	97.72	1.31
5	48	1.98	64.53	20.98	4.73



Scheme 3 Transesterification of *RS*-licarbazepine (**1**) using vinyl benzoate lipase from *Candida rugosa* in MtBE.

Published on 10 October 2016. Downloaded by Norwegian University of Science and Technology on 4/23/2020 8:11:41 AM.

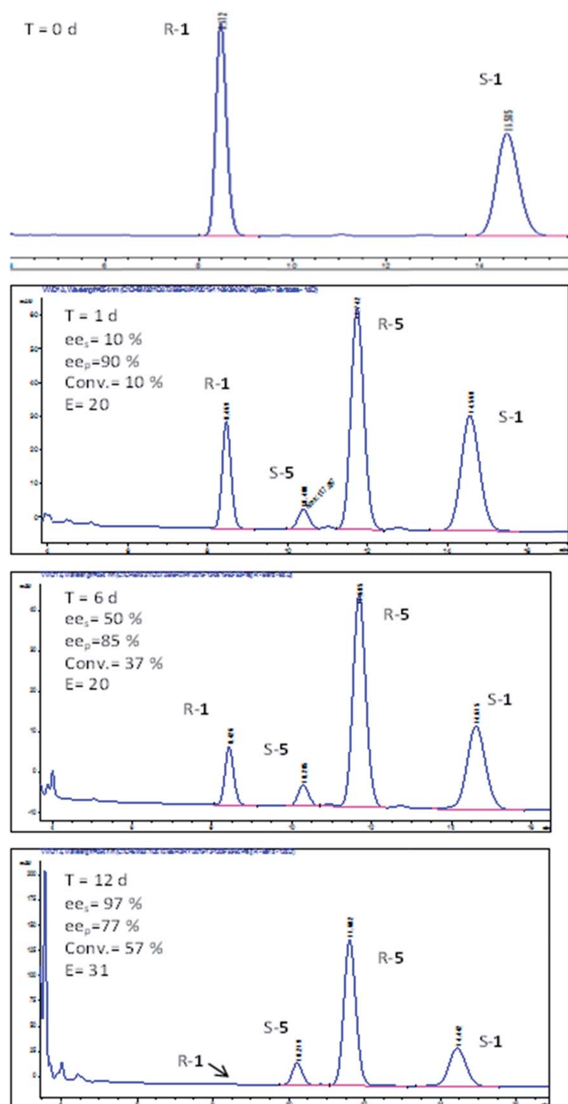


Fig. 4 HPLC traces of large scale transesterification of *RS*-licarbazepine (**1**) using vinyl benzoate lipase from *Candida rugosa* in M*t*BE.

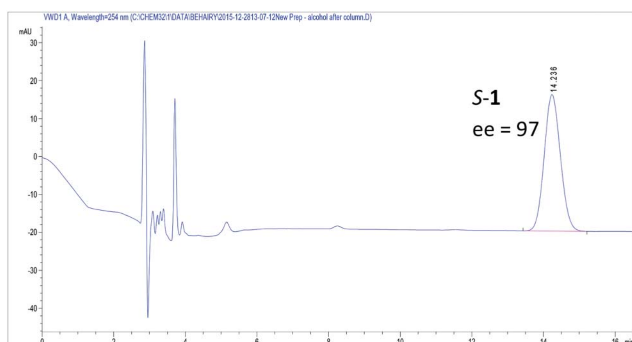


Fig. 5 HPLC traces of enantiopure *S*-licarbazepine (*S*-**1**) after column chromatography (silica, $CHCl_3$/ethyl acetate) using Lux cellulose-2 column and cyclohexane/ethanol 1/1 v/v.

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

Substrate	Docking score (kcal mol^{-1})	E
R-5	−8.540	31.31
S-5	−6.518	31.31
R-4	−6.229	9.54
R-3	−6.034	5.94
S-4	−5.671	9.54
R-2	−5.645	2.35
S-2	−5.223	2.35
S-3	−5.070	5.94

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,[35] 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,[24] this was also evident, as all poses did not penetrate very deep

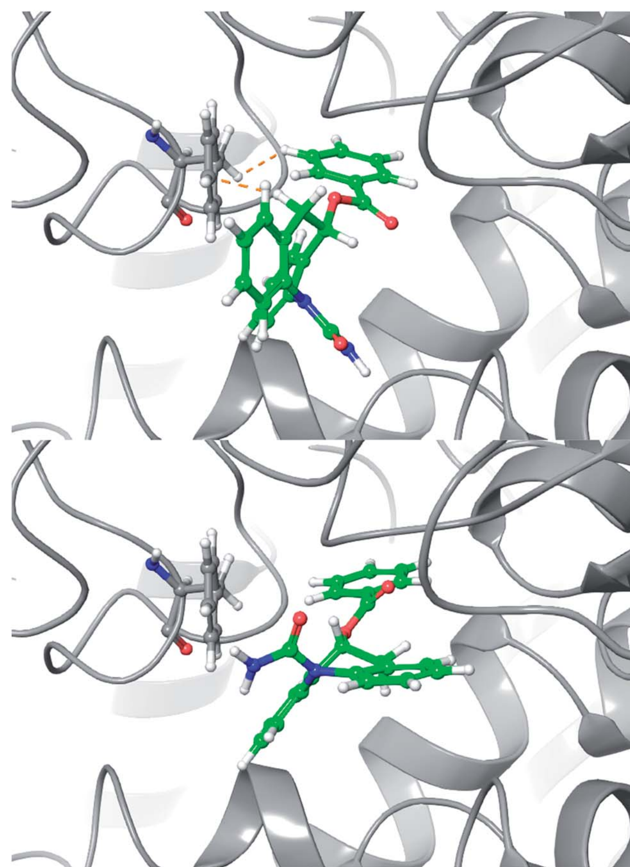


Fig. 6 Best docking poses for substrate *S*-5 (top) and *R*-5 (bottom) on *Candida rugosa* lipase (PDB entry 1LPM). The ligands are shown in green and unfavorable contacts are shown in yellow dotted lines.

This journal is © The Royal Society of Chemistry 2016

Published on 10 October 2016. Downloaded by Norwegian University of Science and Technology on 4/23/2020 8:11:41 AM.

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 enantiomeric 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.[36] 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.[21] Being a biotransformation, the current method provides green access to eslicarbazepine if compared to chemical stereoselective reduction pathways. Further, enantioselective HPLC separation of *RS*-licarbazepine (**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*-licarbazepine with vinyl benzoate in M*t*BE by lipase from *Candida rugosa* afforded *S*-(+)-licarbazepine in good yield and high ee. [$E = 31$, ee $= 97\%$, yield 84%, $\alpha_D^{20} = +105$, c 0.001 g mL^{-1}, CH_3OH]. Docking experiments attributed the enantioselectivity to unfavorable ligand contacts between the *S*-enantiomer of **1** and phenylalanine 296 in the *Candida rugosa* lipase.

Acknowledgements

The authors are grateful for the Egyptian Science and Technology Development Fund for financial supporting of STDF-STF project 11968 (PI: M. F. El-Behairy).

Notes and references

1 T. H. Kim, C. A. Reid and S. Petrou, *Epilepsia*, 2015, **56**, e6–e9.
2 D. Schmidt and C. E. Elger, *Epilepsy Behav.*, 2004, **5**, 627–635.
3 G. Flesch, E. Francotte, F. Hell and P. H. Degen, *J. Chromatogr.*, 1992, **581**, 147–151.
4 K. Hirpara, C. H. Khanduri and M. K. Sharma, WO2013008194 A2, 2013.
5 D. A. Learmonth, WO/2002/092572, 2002.
6 M. M. Gharpure, D. Rane, S. S. Zope, K. B. Narawade and A. A. Thanedar, WO2012156987 A3, 2012.
7 M. N. Bhanu, S. R. F. Crasta and A. V. Joshi, WO/2012/121701, 2012.
8 S. Katkam, R. R. Sagyam, R. Buchikonda, M. M. Muttavarapu, S. Munagala and R. Chikka, WO/2011/091131, 2011.
9 S. J. Desai, A. K. Pandya, S. P. Sawant and K. R. Mehariya, WO/2011/117885, 2011.
10 F. Blatter, D. Grimler, C. Mathes, S. Pfeffer and G. Sedelmeier, WO/2004/031155, 2004.
11 D. A. Learmonth, G. A. Grasa and A. Zanotti-Gerosa, WO/2007/012793, 2007.
12 R. Wisdom, J. Jung and A. Meudt, EP2383261, 2011.
13 C. Daqing and Z. Guoliang, CN102250005, 2011.
14 B. Yu, W. Li and D. Learmonth, WO/2007/117166, 2007.
15 S. Eswaraiah, R. B. Kondal, R. M. Satyanarayana and M. Venkatesh, WO/2011/138795, 2011.
16 S. Biswas, S. K. Dubey, V. Bansal, M. Masand and D. Vir, WO/2012/120356, 2012.
17 A. Gohel, D. Smith, B. Wong, J. Sukumaran, W. L. Yeo, S. J. Collier and S. Novick, WO2012142302A3, 2015.
18 N. K. Modukuru, J. Sukumaran, S. J. Collier, A. S. Chan, A. Gohel, G. W. Huisman, R. Keledjian, K. Narayanaswamy, S. J. Novick, S. M. Palanivel, D. Smith, Z. Wei, B. Wong, W. L. Yeo and D. A. Entwistle, *Org. Process Res. Dev.*, 2014, **18**, 810–815.
19 Z.-M. Ou, H.-B. Shi, X.-Y. Sun and W.-H. Shen, *J. Mol. Catal. B: Enzym.*, 2011, **72**, 294–297.
20 B. Ravinder, S. Rajeshwar Reddy, M. Sridhar, M. Murali Mohan, K. Srinivas, A. Panasa Reddy and R. Bandichhor, *Tetrahedron Lett.*, 2013, **54**, 2841–2844.
21 M. Husain and D. Datta, WO2011045648 (A3) 2011.
22 W. D. R. Schindler, DE2011045 C3, 1979.
23 J. Benes, A. Parada, A. A. Figueiredo, P. C. Alves, A. P. Freitas, D. A. Learmonth, R. A. Cunha, J. Garrett and P. Soares-da-Silva, *J. Med. Chem.*, 1999, **42**, 2582–2587.
24 R. A. Friesner, R. B. Murphy, M. P. Repasky, L. L. Frye, J. R. Greenwood, T. A. Halgren, P. C. Sanschagrin and D. T. Mainz, *J. Med. Chem.*, 2006, **49**, 6177–6196.
25 A. Ghanem, M. N. Aboul-Enein, A. El-Azzouny and M. F. El-Behairy, *J. Chromatogr. A*, 2010, **1217**, 1063–1074.
26 E. Francotte and T. Zhang, *J. Chromatogr. A*, 2016, **1467**, 214–220.
27 D. Sadutto, R. Ferretti, L. Zanitti, A. Casulli and R. Cirilli, *J. Chromatogr. A*, 2016, **1445**, 166–171.
28 J. Shen, T. Ikai and Y. Okamoto, *J. Chromatogr. A*, 2014, **1363**, 51–61.

Published on 10 October 2016. Downloaded by Norwegian University of Science and Technology on 4/23/2020 8:11:41 AM.

29 M. Gumustas, S. A. Ozkan and B. Chankvetadze, *J. Chromatogr. A*, 2016, **1467**, 297–305.
30 M. F. El-Behairy and A. A. El-Azzouny, *J. Liq. Chromatogr. Relat. Technol.*, 2016, **39**, 346–353.
31 A. R. Masters, M. McCoy, D. R. Jones and Z. Desta, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2016, **1015–1016**, 201–208.
32 J. M. Palomo and M. Filice, *Biotechnol. Adv.*, 2015, **33**, 605–613.
33 G. Angajala, P. Pavan and R. Subashini, *Biocatal. Agric. Biotechnol.*, 2016, **7**, 257–270.
34 M. Ahmed, T. Kelly and A. Ghanem, *Tetrahedron*, 2012, **68**, 6781–6802.
35 D. Rotticci, J. Ottosson, T. Norin and K. Hult, in *Enzymes in Nonaqueous Solvents: Methods and Protocols*, ed. E. N. Vulfson, P. J. Halling and H. L. Holland, Humana Press, Totowa, NJ, 2001, vol. 261, pp. 261–276, DOI: 10.1385/1-59259-112-4.
36 R. Piamtongkam, S. Duquesne, F. Bordes, S. Barbe, I. André, A. Marty and W. Chulalaksananukul, *Biotechnol. Bioeng.*, 2011, **108**, 1749–1756.

 This journal is © The Royal Society of Chemistry 2016