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Chemoenzymatic synthesis of luliconazole mediated by lipases

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A straightforward chemoenzymatic synthesis of luliconazole has been developed. The key step involved the preparation of the enantiomerically pure β-halohydrin (1*S*)-2-chloro-1-(2,4-dichlorophenyl)-1-ethanol through kinetic resolution of the corresponding racemic acetate, via hydrolytic approach, mediated by lipase from *Thermomyces lanuginosus* or Novozym 435®. This latter enzyme proved to be a robust biocatalyst in the kinetic resolution, leading to the (*S*)-β-halohydrin with high selectivity (*e.e.* > 99%, E > 200) in just 15 min, at 45 °C, and being reused for five-times with maintenance high values of both conversion and enantioselectivity. Subsequently, the (*S*)-β-halohydrin was subjected to a mesylation reaction and the mesylated derivative reacted with 1-cyanomethylimidazole leading to luliconazole in 43% yield and enantiomeric excess > 99%.

Introduction

Luliconazole (***R*-7**), Scheme 1, also chemically known as (-)-(2*E*)-[(4*R*)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene](1*H*-imidazol-1-yl)acetonitrile, is a pharmaceutically active substance marketed under the brand names Luzu® (Valeant Pharmaceuticals North America) and Lulicon® (Pola Pharma), among others.[1,2] This drug has antifungal action, being used for the treatment of *tinea pedis*, popularly known as athlete's foot, in addition to the treatment against of both candidiasis and pityriasis.[3] *In vitro*, **(*R*)-7** is one of the most potent antifungal agents against filamentous fungi including dermatophytes.[4] Luliconazole was developed in Japan and approved by Japanese health agencies in 2005.[4] More recently, in 2013, the US FDA approved the **(*R*)-7** for the treatment of *tinea pedis* interdigital, *tinea cruris* and *tinea corporis* caused by the microorganisms *Trichophyton rubrum* and *Epidermophyton floccosum*.[5] It is noteworthy that the stereoisomers of luliconazole, (*S*,*E*) and/or *Z*-isomers, are considered impurities and should be removed to obtain the enantiomerically pure active pharmaceutical ingredient **(*R*)-7**.[1] The antifungal action exhibited by **(*R*)-7** resides in the fact that this substance inhibits the enzyme sterol 14α-demethylase (CYP51), which participates in the key step of ergosterol biosynthesis.[6] The mechanism of inhibiton includes the coordination of one of the nitrogen atoms of the imidazole ring with the iron atom of the heme group of the enzyme.[6]

The syntheses of luliconazol have been reported envolving some chiral strategies, such as asymmetric induction in the presence of oxazaborolidines,[1,7] dihydroxylation of Sharpless[7] and chiral catalysts based on ruthenium, iridium or rhodium.[8]

Herein, we report an unprecedented approach to obtain luliconazole, consisting of a lipase mediated chemoenzymatic synthesis, which has the preparation of a chiral β-halohydrin intermediate as key step. The importance of the preparation of chiral β-halohydrins lies in the fact that they are intermediates in the synthesis of substances with high added value.[9,10]

One of the methods used to obtain chiral β-halohydrins is the enzymatic kinetic resolution of the corresponding racemate mediated by lipases.[11] There are some reports of the kinetic resolution of β-halohydrins containing aromatic ring moiety via the acylation reaction, as well as from their corresponding esters, via hydrolytic process. Several lipases have been used in both processes such as *Pseudomonas fluorescens*,[12,13,14] *Burkholderia cepacia*,[15,16,17] *Candida antarctica* type B (Novozym 435®),[13,18,19] *P. cepacia*, [20,21,22,23,24] *C. rugosa*,[21] *Pseudomonas sp.*[25] and *P. aeruginosa*.[26] In some of these examples, the kinetic resolution was performed in the presence of ionic liquids[17,23] or by dynamic kinetic resolution in the presence of ruthenium as racemization agent.[20,26]

Lipases (EC 3.1.1.3) are highlighted as one of the most used enzymes in synthesis of enantiomerically pure drugs since no cofactors are required and regio-, chemo- and enantioselectivities are observed in the resolution process of racemates. Since most of the drugs are chiral, it is important to know which of the stereoisomers has the desired biological activity. Thus, the patient receives only one dose of the active stereoisomer, avoiding the side effects caused by the undesired stereoisomer. Therefore, biocatalysis represents an alternative tool for the production of enantiomerically pure chiral drugs over conventional chemical processes.[27]

In this paper, we describe the chemoenzymatic synthesis of luliconazole **(*R*)-7**, which had as key step the preparation of the chiral β-halohydrin (1*S*)-2-chloro-1-(2,4-dichlorophenyl)-1-ethanol **(*S*)-2**, via kinetic resolution of acetate ***rac*-3**, mediated by a lipase, Scheme 1. Our focus was to perform an optimization of the reactional conditions, including the enzymes recycling outcome.

Results and Discussion

The chemoenzymatic synthesis of luliconazole is depicted in Scheme 1. The key step was the kinetic enzymatic resolution of the racemic 2-chloro-1-(2,4-dichlorophenyl)ethyl acetate (***rac*-3**), via a hydrolytic approach, mediated by a lipase.

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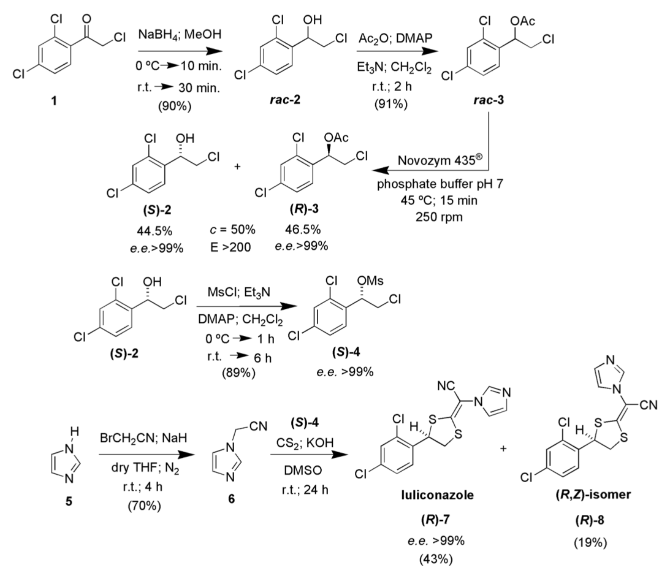


Scheme 1. Chemoenzymatic synthesis of luliconazole.

Synthesis of racemic halohydrin (*rac*-2) and its acetate (*rac*-3)

As first step, the chemical reduction of the α-chloroketone **1** was carried out by using sodium borohydride (0.5 eq.) in MeOH[28] to yield ***rac*-2** in 90% yield. Next, the chemical acetylation of ***rac*-2** was performed using Ac_2O, DMAP and Et_3N at room temperature for 2 h[29] and allowed the preparation of ***rac*-3** with 91% isolated yield, Scheme 1. Adequate chiral GC analyses were developed for both ***rac*-2** and ***rac*-3** in order to achieve a reliable method to measure the enantiomeric excesses of both remaining substrate and the final product from the lipase-catalyzed resolution, Fig. 1.

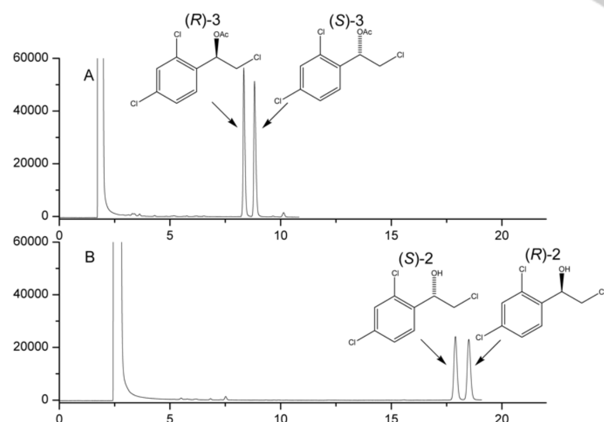


Figure 1. A) GC chromatogram of *rac*-2-chloro-1-(2,4-dichlorophenyl)ethyl acetate (***rac*-3**: (*R*) t_R 8.33 min; (*S*) t_R 8.82 min). B) GC chromatogram of *rac*-2-chloro-1-(2,4-dichlorophenyl)1-ethanol (***rac*-2**: (*S*) t_R 17.90 min.; (*R*) t_R 18.50 min).

Screening of lipase-mediated kinetic resolution of acetate *rac*-3 via hydrolysis

For initial screening of lipases, twelve commercially available enzymes (ratio 2:1 in weight respect to ***rac*-3**) were tried in the resolution of ***rac*-3** at 30 °C, 250 r.p.m., phosphate buffer pH 7 (0.1M). Reactions were monitored for a maximum of 24 h and stopped before this time if the conversion had reached a value close to 50%. The product β-halohydrin **(*S*)-2** and the remaining acetate **(*R*)-3** were obtained, and the results are summarized in Table 1.

Table 1. Kinetic resolution of ***rac*-3**, via hydrolysis using lipases.[a]

Entry	Lipase	Time (h)	e.e.s (%)[b]	e.e.p (%)[b]	c (%)[c]	E[d]
1	*P. fluorescens*	19	77	70	52	13
2	*P. camemberti*	24	3	3	50	1
3	*R. miehei* immobilized on anionic resin	14	60	57	51	6
4	Amano lipase PS from *B. cepacia* immobilized on diatomaceous earth	19	73	66	52	10
5	Amano lipase PS from *B. cepacia*	19	80	74	52	16
6	TLL[e]	24	>99	92	52	128
7	Novozym 435®	24	97	95	50	185
8	Amano lipase from *M. javanicus*	24	11	59	16	4
9	*C. rugosa*	24	9	48	16	3
10	Porcine pancreas	24	3	3	50	1
11	*R. niveus*	24	1	64	2	5
12	*R. oryzae* immobilized on immobead-150	19	53	57	48	6

[a] Conditions: 30 °C, lipase:***rac*-3** (2:1) at 250 r.p.m. [b] Determined by GC. [c] Conversion, $c = e.e._s/(e.e._s + e.e._p)$. [d] Enantiomeric ratio, $E = \ln[1 - c(1 + e.e._p)]/\ln[1 - c(1 - e.e._p)]$. [e] TLL: *T. lanuginosus* immobilized on immobead-150.

Among the lipases evaluated in the hydrolysis reaction of ***rac*-3**, *Thermomyces lanuginosus* immobilized on immobead-150 (TLL) and CAL-B (Novozym 435®) were the enzymes that provided better results. Conversions of 52 and 50%, and E values of 128 and 185 were observed, respectively, in 24 h of reaction

(Table 1, entries 6 and 7). On the other hand, lipase from *Rhizomucor miehei* immobilized on anionic resin was the enzyme with the highest activity in the kinetic resolution of ***rac*-3**, with a conversion of 51% in only 14 h, but with low selectivity (E value of 6), Table 1 entry 3. Lipase from *Rhizopus niveus* was the less active enzyme, leading to a conversion of only 2% in 24 h of reaction, (Table 1, entry 11).

The remainder of the lipases evaluated, as *P. fluorescens*, *Penicillium camemberti*, Amano PS from *B. cepacia* immobilized on diatomaceous earth, Amano PS from *B. cepacia*, Amano lipase from *Mucor javanicus*, *C. rugosa*, porcine pancreas and *Rhizopus oryzae* immobilized on immobead-150, led to conversions of about 50%, but with low enantioselectivity (E values ranging from 1 to 16; Table 1 entries 1, 2, 4, 5, 8, 9, 10 and 12, respectively).

Improving the kinetic resolution of *rac*-3 through temperature variation

In order to improve enzymatic resolution of ***rac*-3**, we performed a detailed study of the reaction condition, varying some parameters, such as temperature, reaction time and the ratio enzyme/substrate (w/w). For this study, we selected the enzymes TLL immobilized on immobead-150 and Novozym 435® that were most promising in the kinetic resolution of ***rac*-3** (Table 1, entries 6 and 7). In addition, since these two lipases are commercially immobilized, reuse studies have been performed.

In order to reduce the reaction time, the first variable to be changed was the temperature. With additions of 5 °C, the kinetic resolution of ***rac*-3** was evaluated at 35, 40 and 45 °C. The results were summarized in Table 2.

Table 2. Results of kinetic resolution of ***rac*-3**, via hydrolysis, mediated by lipases, varying temperature and enzyme/substrate ratio (w/w).

Entry	Lipase	T(°C)	Time (min)	$e.e._s$ (%)[a]	$e.e._p$ (%)[a]	c (%)[b]	E[c]
1[d]	Novozym 435®	35	960	>99	>99	50	>200
2[d]	Novozym 435®	40	120	>99	>99	50	>200
3[d]	Novozym 435®	45	15	>99	>99	50	>200
4[e]	Novozym 435®	45	15	>99	>99	50	>200
5[f]	Novozym 435®	45	15	76	>99	43	>200
6[d]	TLL[g]	35	960	97	96	50	>200
7[d]	TLL[g]	40	120	>99	>99	50	>200
8[d]	TLL[g]	45	15	>99	>99	50	>200
9[h]	TLL[g]	45	15	>99	>99	50	>200
10[i]	TLL[g]	45	15	41	>99	30	>200

[a] Determined by GC. [b] Conversion, $c = e.e._s/(e.e._s + e.e._p)$. [c] Enantiomeric ratio, $E = \ln[1 - c(1 + e.e._p)]/\ln[1 - c(1 - e.e._p)]$. [d] lipase:***rac*-3** (2:1). [e] lipase:***rac*-3** (0.5:1). [f] lipase:***rac*-3** (0.25:1). [g] TLL: *T. lanuginosus* immobilized on immobead-150. [h] lipase:***rac*-3** (1.5:1). [i] lipase:***rac*-3** (1:1).

As we increased the temperature by 5 °C (from 30 to 35 °C), there was a decrease in reaction time from 24 to 16 h for both Novozym 435® and TLL enzymes, Table 2, entries 1 and 6. However, with Novozym 435® we observed an increase in the enantiomeric excess values for both product and remaining substrate (> 99%), Table 2, entry 1. No significant changes in the enantiomeric excess values of product and remaining substrate were observed for TLL, Table 2, entry 6. On the other hand, the increment of 5 °C in the temperature (from 35 to 40 °C) led to a significant decrease in reaction time from 16 to 2 h (Table 2, entries 2 and 7). In this case, both lipases yielded the product and the remaining substrate with enantiomeric excess > 99%. A surprising result was obtained when the kinetic resolution was performed at 45 °C. At this temperature, the reaction time for an ideal kinetic resolution was only 15 min and both lipases led to the product and remaining substrate with enantiomeric excess > 99%, conversion of 50% and E value > 200 (Table 2, entries 4 and 8).

Once the ideal temperature (45 °C) was determined, at which the kinetic resolution of ***rac*-3** occurred in the shortest possible time (15 min), we decided to evaluate the decrease of the loaded enzyme on the reaction.

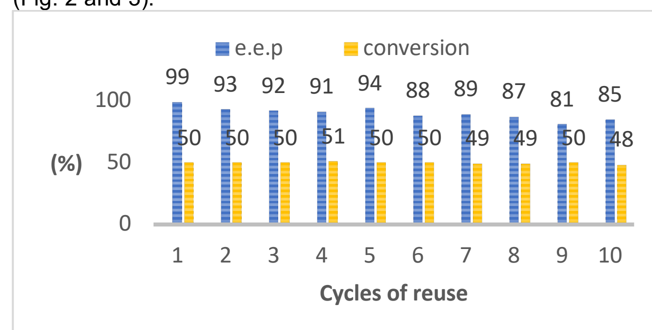
Effect of enzyme load in the kinetic resolution of *rac*-3

For Novozym 435®, the ratio enzyme:substrate was gradually decreased (from 2:1 to 0.25:1) and the kinetic resolution of ***rac*-3** remained efficient up to 0.5:1 (w:w). Enantiomeric excess > 99%, 50% conversion and E value > 200 was observed for both product and substrate, Table 2, entry 4. However, with an enzyme:substrate ratio of 0.25:1, the conversion had a slight decrease (43%) leading to an enantiomeric excess of substrate remaining of 76%, Table 2, entry 5. For TLL to maintain an optimal kinetic resolution of ***rac*-3**, the lowest enzyme:substrate ratio was 1.5:1, Table 2, entry 9. When we decreased the ratio to 1:1, the conversion showed a decrease (30%) and the enantiomeric excess of the substrate was only 41%, Table 2, entry 10.

Finally, since the most efficient enzymes (Novozym 435® and TLL) in the kinetic resolution of ***rac*-3** are commercially immobilized, we decided to carry out a study of their reuse in ten reaction cycles.

Reuse of the immobilized lipases

This study was carried out in order to investigate the efficiency of Novozym 435® and TLL in maintaining satisfactory conversion and enantioselectivity values up to ten cycles of reuse (Fig. 2 and 3).



[a]Conditions: phosphate buffer pH 7 (0.1M), 45 ºC, lipase:***rac*-3a** (0.5:1) and 15 min at 250 rpm.

Figure 2. Results from the reuse of Novozym 435®.[a]

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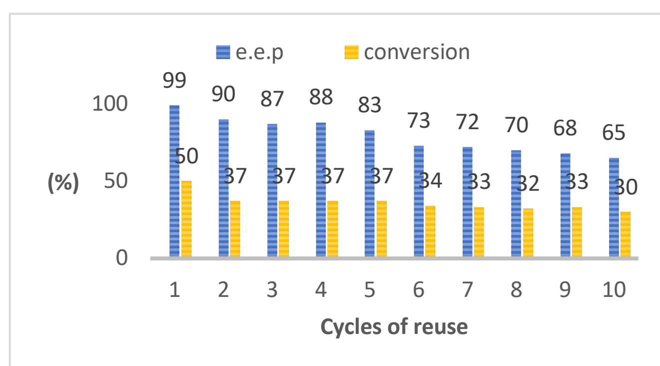

[a]Conditions: phosphate buffer pH 7 (0.1M), 45 ºC, lipase:***rac-3a*** (1.5:1) and 15 min at 250 rpm.

Figure 3. Results from the reuse of TLL immobilized on immobead-150.[a]

Conversion values close to 50% were observed for Novozym 435® during ten reused cycles. Additionally, it was obtained enantiomeric excess values of product greater than 90% up to the fifth reaction cycle. From the sixth reaction cycle, the enzyme lost some of its selectivity, with enantiomeric excess values of product varying between 81 and 89% (Fig. 2).

When the reuse study was performed with TLL, it was verified that in the second reaction cycle the activity decreased to 37%, a value that remained constant until the fifth reaction cycle. In the next reaction cycles, there was a slight decrease in the conversion values, culminating in a conversion of 30% in the last cycle. Concerning the selectivity, it was observed that the enantiomeric excess of the product decreased to 90% in the second reaction cycle and continued progressively decreasing until the tenth cycle at a value of 65% (Fig. 3).

Once the enzymatic loading and reuse studies were performed, it was possible to verify that Novozym 435® presented more advantages compared to TLL. In fact, Novozym 435® required a lower enzyme:substrate ratio (0.5:1) in detriment of 1.5:1 to TLL. Moreover, Novozym 435® can be reused in up to five reaction cycles maintaining high values of selectivity and conversion. On the other hand, in the presence of TLL, the conversion showed a considerable decrease already in the second reaction cycle, followed by a progressive decrease in the selectivity in the subsequent reaction cycles.

After optimizing the enzymatic kinetic resolution of ***rac*-3**, we proceed with the following steps to obtain luliconazole **(*R*)-7**.

Synthesis of luliconazole (*R*)-7

The kinetic resolution of ***rac*-3** was performed under the optimized conditions (Scheme 1), phosphate buffer (pH 7), Novozym 435®, enzyme:substrate (0.5:1), 45 °C and 15 min of reaction, and provided the β-halohydrin **(*S*)-2** and the remaining acetate **(*R*)-3** with enantiomeric excess values > 99%, conversion of 50% and enantioselectivity (E) > 200.

Posteriorly, the **(*S*)-2** was subjected to a mesylation reaction with MsCl, DMAP, Et_3N in CH_2Cl_2 for 7 h to give the mesylated product **(*S*)-4** in 89% yield and enantiomeric excess >99%, Scheme 1.[30]

Concomitantly, 1-cyanomethylimidazole **6** was obtained in 70% yield from the reaction of imidazole **5** with bromoacetonitrile, NaH in dry THF for 4 h, Scheme 1.[31]

Finally, in the last step, the mesylate **(*S*)-4** was subjected to the reaction with 1-cyanomethylimidazole **6** in the presence of CS_2, K_2CO_3 in DMF, at room temperature and 24 h of reaction.[32] However, under such conditions the substrate **(*S*)-4** was recovered unchanged from the reaction medium. In search of better reaction conditions, we decided to use KOH as base and DMSO as the solvent. In this case, we obtained a mixture of the *E* (***R*-7**) and *Z* (***R*-8**) isomers in a ratio of 2:1. After purification, the luliconazole (*E*-isomer) was obtained in 43% yield and enantiomeric excess > 99%, Scheme 1.[6]

Conclusions

In summary, we prepared luliconazole via an original approach involving the chemoenzymatic process using lipases. Both lipases TLL immobilized on immobead-150 and Novozym 435® were able to resolve the racemic 2-chloro-1-(2,4-dichlorophenyl)ethyl acetate (***rac*-3**) via a hydrolytic process. The enantiomerically pure key intermediate β-halohydrin (1*S*)-2-chloro-1-(2,4-dichlorophenyl)-1-ethanol **(*S*)-2** was obtained with conversion of 50% in only 15 min of reaction. Novozym 435® stood out as the best lipase because it required a lower enzymatic load enzyme:substrate (0.5:1) compared to TLL (1.5:1). Moreover, Novozym 435® proved to be a robust biocatalyst, since it could be reused in five reaction cycles, maintaining high values of both conversion and enantioselectivity. Finally, this unprecedented approach to obtain luliconazole can be considered eco-friendly, since a stable, low cost, reusable, commercially available and highly enantioselective biocatalyst was used.

Experimental Section

Enzymes

(i) Immobilized lipases: *C. antarctica* lipase type B immobilized on acrylic resin (CAL-B, Novozym 435, 7,300.0 U/g) and *R. miehei* lipase immobilized on anionic resin (RML, 150.0 U/g) were purchased from Novozymes®. *B. cepacia* lipase immobilized on diatomaceous earth (Amano PS-IM, ≥500 U/g), *T. lanuginosus* lipase immobilized on immobead-150 (TLL, 250.0 U/g) and *R. oryzae* lipase immobilized on immobead-150 (ROL, 340.0 U/g) were acquired from Sigma-Aldrich®. (ii) Crude lipase preparations: *M. javanicus* lipase (Amano M, ≥10,000 U/g), *R. niveus* lipase (RNL, 1,5 U/mg), *B. cepacia* lipase (Amano PS, ≥30,000 U/g), *P. fluorescens* lipase (AK, 22,100.0 U/g), *P. camemberti* lipase (Amano G, 50.0 U/g) were acquired from Sigma-Aldrich®. Porcine pancreas lipase (PPL, 46.0 U/g solid), and *C. rugosa* lipase (CRL, 1.4 U/g) were obtained from Sigma-Aldrich®.

Chemical Materials

Chemical reagents were purchased from different commercial sources and used without further purification. Methanol, DMF, chloroform, DMSO, ethyl acetate and dichloromethane were acquired from Synth®. Tetrahydrofuran and carbon disulfide were acquired from Sigma-Aldrich®. Solvents were distilled over an adequate desiccant under nitrogen. Analytical TLC analyses were performed on aluminum sheets pre-coated with silica gel 60 F254 (0.2 mm thick) from Merck®. Flash chromatographies were performed using silica gel 60 (230-240 mesh).

Analysis

Melting points were determined in open capillary tube Microquímica model MQAPF-302 and are uncorrected. ^{1}H and ^{13}C NMR were obtained using Spectrometer Bruker model Avance DPX 300, operating at frequency of 300 MHz for hydrogen and frequency of 75 MHz for carbon. The chemical shifts are given in delta (*δ*) values and the coupling constants (*J*) in Hertz (Hz). Measurement of the optical rotation was done in a Jasco P-2000 polarimeter. Gas chromatograph (GC) analyses were carried out in a Shimadzu chromatograph model GC 2010 with a flame ionization detector using a chiral column CP-chirasil-dex (25 m x 0.25 mm x 0.25 µm, 0.5 bar N_2). For the following of the reaction time courses: *rac*-2-chloro-1-(2,4-dichlorophenyl)ethyl acetate (***rac*-3**): 185 ºC; 1 ºC/min. 190 ºC (hold 5 min.); 0.5 ºC/min. 200 ºC (hold 15 min.). Retention times were: (*R*)-acetate= 8.33 min.; (*S*)-acetate= 8.82 min. Haloydrin ***rac*-2** was analyzed using a chiral column Rt®bDEXcst (30 m x 0.39 mm x 0.25 µm, 0.5 bar N_2). For the following of the reaction time courses: *rac*-2-chloro-1-(2,4-dichlorophenyl)ethanol (***rac*-2**): 185 ºC; 1 ºC/min. 190 ºC (hold 5 min.); 0.5 ºC/min. 200 ºC (hold 15 min.). Retention times were: (*S*)-halohydrin= 17.90 min.; (*R*)-halohydrin= 18.50 min.

Synthesis of *rac*-β-halohydrin (*rac*-2)

A mass of 2-chloro-1-(2,4-dichlorophenyl)ethanone (**1**) (1 g, 4.5 mmol) was dissolved in MeOH (40 mL). Then, $NaBH_4$ (0.085 g, 2.2 mmol) of was added slowly at 0 °C. The reaction was stirred for 30 min at room temperature. Upon completion, the MeOH was evaporated under reduced pressure. Then, 1 M HCl (10 mL) of was added followed by extraction with EtOAc (4 x 60 mL). Organic phases were combined and dried over anhydrous Na_2SO_4, filtered and solvent was evaporated under reduced pressure. Then, the crude product was purified by performing column chromatography, employing silica gel and $CHCl_3$ to afford *rac*-β-halohydrin ***rac*-2** in 90% yield (0.913 g, 4.0 mmol).

Synthesis of *rac*-acetate (*rac*-3)

A suspension of DMAP (0.054 g, 0.4 mmol) and acetic anhydride (1.25 mL, 13.3 mmol) was dissolved in CH_2Cl_2 (40 mL). Then, ***rac*-2** (1 g, 4.4 mmol) and triethylamine (616.3 µL, 4.4 mmol) were added. The reaction was stirred for 2 h at room temperature. After this time, distilled water (10 mL) was added followed by extraction with CH_2Cl_2 (3 x 50 mL). The organic phases were combined and dried over anhydrous Na_2SO_4. Then, after filtration, the solvent was evaporated under reduced pressure and the crude product was purified using a silica gel column chromatography and $CHCl_3$ to afford *rac*-acetate ***rac*-3** in 91% yield (1.071 g, 4.0 mmol).

Synthesis of (1*S*)-2-chloro-1-(2,4-dichlorophenyl)-2-methanesulfonate (*S*-4)

A mass of **(*S*)-2** (0.5 g, 2.2 mmol) of was dissolved in CH_2Cl_2 (25 mL) at 0 °C. Then, triethylamine (307 µL, 2.2 mmol), DMAP (0.027 g, 0.2 mmol) and MsCl (855 µL, 11.0 mmol) were added in 0 ºC for 1 h. Then, the reaction mixture was stirred at room temperature for 6 h. After this time, 20 mL of distilled water was added followed by extraction with CH_2Cl_2 (3 x 40 mL). Then, the organic phase were combined and treated with saturated aqueous $NaHCO_3$ solution (20 mL) and the organic phase was collected and dried with anhydrous Na_2SO_4. Finally, after filtration, the solvent was evaporated under reduced pressure and the crude product was purified using column chromatography with silica gel ($CHCl_3$/hexane 7:3) to afford **(*S*)-4** as yellow solid in 89% yield (0.607 g, 2.0 mmol).

Synthesis of 1-cyanomethylimidazole (6)

To a solution of imidazole (**5**) (0.5 g, 7.3 mmol) in dry THF (50 mL) under nitrogen atmosphere, NaH (0.176 g, 7.3 mmol) was added until complete release of hydrogen. After, bromoacetonitrile (510 µL, 7.3 mmol) was added dropwise, with the appearance of a yellow color in the reaction. The reaction mixture was stirred for 4 h at room temperature. Then, the solvent was evaporated under reduced pressure and distilled water (10 mL) was added followed by extraction with CH_2Cl_2 (3 x 50 mL). The organic phase was dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated under reduced pressure. Then, the crude product was purified by performing column chromatography on silica gel ($CHCl_3$/MeOH 9.5:0.5) to afford (**6**) as a brown solid in 70% yield (0.546 g, 5.1 mmol).

Synthesis of luliconazole (*R*-7)

A mass of KOH (0.210 mg, 3.7 mmol) was dissolved in DMSO (3 mL). Then, **6** (0.1 g, 0.9 mmol) and carbon disulfide (56 µL, 0.9 mmol) in of DMSO (3 mL) at 4 ºC were added. The reaction was stirred for 2 h at room temperature to form the dithiolate salt. Then, **(*S*)-4** (0.560 g, 1.9 mmol) was added slowly at 4 °C and the reaction was stirred for 24 h at room temperature. After this time, cold water (30 mL) was added and the reaction mixture was stirred for 10 min. Then, the product was extracted with EtOAc (3 x 40 mL) and the organic phase were washed with ice water (2 x 20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified using column chromatography with silica gel ($CHCl_3$/CH_3CN 9:1) to afford the (*R*,*Z*)-isomer with 19% yield (0.062 g, 0.18 mmol) and **(*R*)-7** as yellow solid in 43% yield (0.141 g, 0.4 mmol).

General procedure for the lipase-catalyzed hydrolysis of *rac*-3

A suspension of ***rac*-3** (0.030 g, 0.1 mmol) and lipase (ratio 2:1 in weight respect to the ***rac*-3**) in phosphate buffer 100 mM pH 7.0 (1.0 mL) was shaken in their proper temperature and time reaction at 250 rpm. After the conversion reaches a value close to 50%, the products were extracted with EtOAc (3 x 5 mL). The organic phases were combined and dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The reaction crude was purified by flash chromatography on silica gel (100% $CHCl_3$), yielding (*R*)-acetate (***R*-3**) and (*S*)-halohydrin (***S*-2**) being their enantiomeric excess determined by GC.

Synthesis of (*R*)-3 and (*S*)-2 mediated by Novozym 435®

A suspension of ***rac*-3** (0.5 g, 1.9 mmol) and 0.25 g of Novozym 435® in phosphate buffer 100 mM pH 7.0 (19 mL) was shaken in 45 ºC and 15 min at 250 rpm. After the conversion has reached a value close to or 50%, the products were extracted with EtOAc (3 x 20 mL). The organic phases were combined and dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The reaction crude was purified by flash chromatography on silica gel and $CHCl_3$, yielding 46.5% of (*R*)-acetate (***R*-3**) (0.454 g, 1.7 mmol) with $[\alpha]_D^{23}$=-52.3 (*c*=1.0 in ethyl acetate) for 99% *e.e.* and 44.5% of (*S*)-halohydrin (***S*-2**) (0.383 g, 1.7 mmol) with $[\alpha]_D^{20}$=+51.1 (*c*=2.50 in chloroform) for 99% *e.e.*; Lit $[\alpha]_D^{25}$=-52.8 (*c*=2.55 in chloroform) for 99% *e.e.* of the (*R*)-enantiomer.[33]

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rac-2-chloro-1-(2,4-dichlorophenyl)ethanol (***rac*-2**): 90% yield. Orange solid. R_f=0.45 ($CHCl_3$); m.p. 47-49 C; Lit m.p. 46-47 C;[15] ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ=2.50 (br s, OH, 1H), 3.52 (dd, J=11.8, 5.0 Hz, 1H), 3.87 (dd, J=8.3, 2.0 Hz, 1H), 5.26 (dd, J=6.0, 2.7 Hz, 1H), 7.31 (dd, J=10.1, 2.3 Hz, 1H), 7.38 (d, J=1.8 Hz, 1H), 7.57 ppm (d, J=8.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ=49.4 (CH_2), 70.5 (CH), 127.8 (CH), 128.7 (CH), 129.5 (CH), 132.7 (C), 134.8 (C), 136.1 ppm (C).

rac-2-chloro-1-(2,4-dichlorophenyl)ethyl acetate (***rac*-3**): 91% yield. Yellow liquid. R_f=0.61 ($CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ=2.17 (s, 3H), 3.72 (dd, J=11.8, 4.9 Hz, 1H), 3.84 (dd, J=11.8, 8.1 Hz, 1H), 6.31 (dd, J=10.6, 3.7 Hz, 1H), 7.28 (dd, J=10.5, 2.1 Hz, 1H), 7.37 (d, J=8.4 Hz, 1H), 7.40 ppm (d, J=1.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ=21.0 (CH_3), 45.2 (CH_2), 71.4 (CH), 127.7 (CH), 128.9 (CH), 129.8 (CH), 133.2 (C), 133.7 (C), 1353 (C), 169.6 ppm (C).

(*S*)-2-chloro-1-(2,4-dichlorophenyl)-2-methanesulfonate (***S*-4**): 89% yield. Yellow solid. R_f=0.45 ($CHCl_3$/hexane 7:3); m.p. 73-76 C; $[\alpha]_D^{20}$=+64.0 (c=1.0 in chloroform) for 99% *e.e.*; ^{1}H NMR (300 MHz, $CDCl_3$ TMS): δ=3.09 (s, 3H), 3.75 (dd, J=12.1, 4.7 Hz, 1H), 3.84-3.87 (m, 1H); 6.05 (dd, J=9.3, 3.5 Hz, 1H), 7.27-7.32 (m, 1H), 7.34-7.38 (m, 1H), 7.51-7.59 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ=38.9 (CH_3), 45.3 (CH_2), 78.7 (CH), 128.1 (CH), 129.1 (CH), 129.9 (CH), 132.4 (C), 132.7 (C), 136.2 ppm (C).

1-cyanomethylimidazole (**6**): 70% yield. Brown solid. R_f=0.50 ($CHCl_3$/MeOH 9.5:0.5); m.p. 141-142 C; Lit m.p. 138 C;[34] ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ=4.92 (s, 1H), 7.10 (s, 1H), 7.27 (s, 1H), 7.55 ppm (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ=34.5 (CH_2), 113.8 (C), 119.1 (CH), 130.9 (CH), 137.2 ppm (CH).

(*R*,*Z*)-Isomer (***R*-8**): 19% yield. Yellow solid. R_f=0.41 ($CHCl_3$/CH_3CN 9:1); m.p. 113-115 C; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ=3.74 (dd, J=18.0, 6.0 Hz, 1H), 3.96 (dd, J=15.0, 3.0 Hz, 1H), 5.63 (dd, J=15.0, 6.0 Hz, 1H), 7.06 (s, 1H), 7.17 (s, 1H), 7.27-7.33 (m, 1H), 7.46 (d, J=1.0 Hz, 1H), 7.52 (d, J=9.0 Hz, 1H), 7.65 ppm (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ=44.2 (CH_2), 55.6 (CH), 77.6 (C), 114.6 (C), 119.3 (CH), 128.1 (CH), 129.2 (2CH), 130.4 (2CH), 131.4 (C), 134.4 (C), 136.1 (C), 137.2 ppm (CH).

Luliconazole (***R*-7**): 43% yield. Yellow solid. R_f=0.45 ($CHCl_3$/CH_3CN 9:1); m.p. 151-152 C; Lit m.p. 151.1 C;[35] $[\alpha]_D^{20}$=-50.7 (c=1.0 in dimethylformamide) for 99% *e.e.*; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ=3.66 (dd, J=11.6, 4.4 Hz, 1H), 3.89 (dd, J=10.3, 3.1 Hz, 1H), 5.70 (dd, J=7.6, 3.2 Hz, 1H), 7.06 (s, 1H), 7.19 (s, 1H), 7.35 (dd, J=6.1, 1.1 Hz, 1H), 7.49 (d, J=1.1 Hz, 1H), 7.59 (d, J=5.0 Hz, 1H), 7.66 ppm (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ=42.8 (CH_2), 44.4 (CH), 77.4 (C), 114.7 (C), 119.4 (CH), 128.2 (CH), 129.2 (CH), 130.3 (2CH), 130.4 (C), 131.5 (C), 134.4 (CH), 136.1 (C), 137.2 ppm (C).

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Conflict of interest

The authors declare no conflict of interest.

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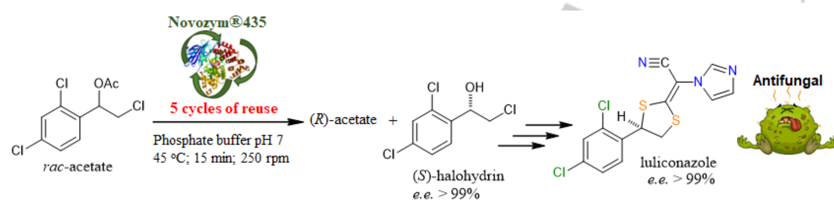

A new approach based on biocatalysis was developed for the production of the potent antifungal luliconazole. The lipase Novozym 435® proved to be a robust biocatalyst in the key step involving the preparation of an enantiomerically pure β-halohydrin in only 15 min of reaction. The enzyme was reused in up to 5 reaction cycles with maintenance of high values of activity and selectivity. After a few more steps, luliconazole was obtained in good yield and enantiomeric excess.

*Thiago de S. Fonseca, Lara D. Lima, Maria da C. F. de Oliveira, Telma L. G. de Lemos, Davila Zampieri, Francesco Molinari, Marcos C. de Mattos**

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Luliconazole via biocatalytic process

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