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Resolution of racemic amines via lipase-catalyzed benzoylation: Chemoenzymatic synthesis of the pharmacologically active isomers of labetalol

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ABSTRACT

Lipase-catalyzed benzoylation of amines was shown to be feasible, in some cases with high enantioselectivity, and the best results were obtained using immobilized lipase from *Candida antarctica* (Novozym 435) and methyl benzoate as acyl donor in the presence of molecular sieves. The procedure was optimized for the resolution of (±)-1-methyl-3-phenylpropylamine, a key intermediate in the synthesis of antihypertensive drug labetalol, and the enantiopure (*R*)-benzamide was then converted into the pharmacologically active isomers of the drug. In comparison with the reported synthesis of chiral isomers of labetalol, this chemoenzymatic route offers the advantage in the lack of any chiral stoichiometric auxiliary.

1. Introduction

Biocatalysis is nowadays a well-accepted methodology for the preparation of pure isomers of chiral drugs providing a clean, economical and sustainable alternative to conventional chemical processes. In this context, lipases have received growing interest for their remarkable characteristics of regio-, chemo- and enantioselectivity in the resolution process of racemates [1,2] as well as in the desymmetrization of prochiral substrates [3], without the use of cofactors, and for their excellent stability in organic solvents, that facilitate the solubilization of organic substrates and makes transesterification reactions feasible.

Many chiral drugs or intermediates for the synthesis of pharmaceuticals contains secondary alcohol groups and most of the reported lipase-catalyzed processes deal with the kinetic resolution of such alcohols through enantioselective transesterification in organic solvent and a large structural diversity is well tolerated [4,5].

Although amine and/or amide groups are present in many pharmacologically active compounds, the kinetic resolution of amine drugs or intermediates by lipase-catalyzed acylation is still limited [6,7] and enantiopure amines are usually obtained by differential crystallization of their diastereoisomeric salts with chiral acids [8,9] or by asymmetric synthesis [10]. However, the development of effective lipase-catalyzed acylation of amines could overcome the need of stoichiometric chiral reagents or expensive and synthetically demanding catalysts.

Lipase from *Candida antarctica* has proved the most effective catalyst for the enantioselective acylation of amines [11], with a special focus on (±)-1-phenylethylamine and related α-branched primary amines. Esters of alkylcarboxylic acids [12], *O*-methoxyesters [13] or dialkylcarbonates [14] are usually employed as acyl donors, since vinyl esters, the best choice in the enzymatic resolution of alcohols, are not suitable for amines due to their high reactivity that promotes spontaneous side reactions.

Since in the enzymatic resolution process one enantiomer of the amine substrate is converted into the corresponding amide, wherefrom it can be recovered by acid hydrolysis in some cases using harsh conditions, the search of alternative acyl donors could be valuable.

In spite of the interest in benzamides [15–17], that can be easily converted into *N*-benzyl protected amines for further synthetic manipulations and/or into the corresponding free amines by simple hydrogenolysis, enzymatic aminolysis reactions of benzoate esters have not previously reported.

Labetalol **1** (Fig. 1) is an antihypertensive drug with dual α_1- and β_1-adrenoceptor antagonist activities [18] commercially available as an equimolecular mixture of the four possible isomers. It has been shown that the *SS*- and *RS*- isomers are inactive, whereas the most α_1-blocking activity is due to the *S,R*-enantiomer and the *R,R*-enantiomer displays the highest potency in the β_1- adrenoceptor blocking activity [19]. Dilevalol (*R,R*)-**1** never reached the market owing to its hepatoxicity not seen to the same extent with labetalol, while the combination of *RR*- and *SR*- isomers, with a shared *R*-configuration of the aminic carbon, may be a valuable substitute for the current drug in the treatment of systemic hypertension [20].

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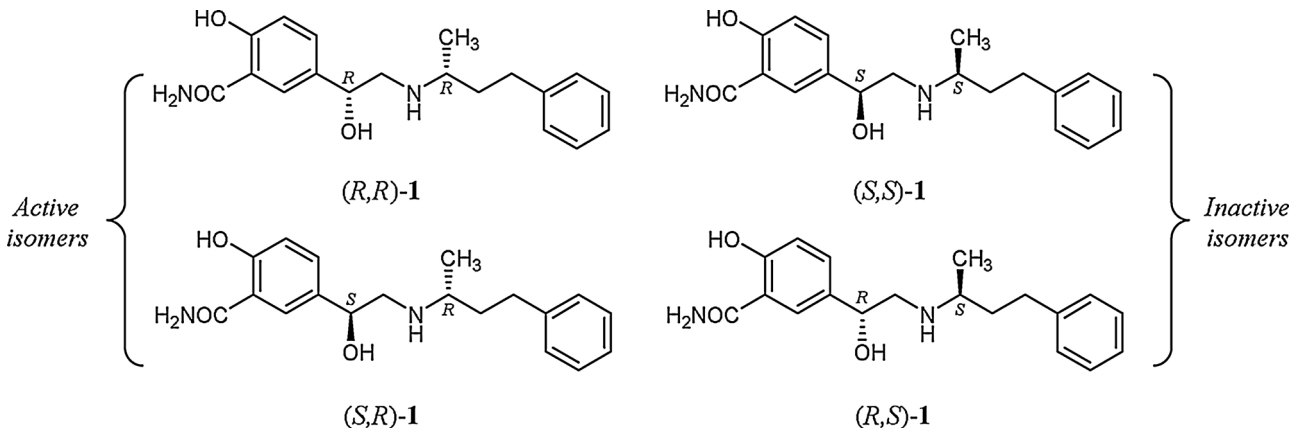

Fig. 1. Chemical structures of the four stereoisomers of labetalol drug.

Since the reported synthetic routes to single enantiomers of labetalol start from *N*-benzyl protected amine **2a** [18] or **2b** [19,21] (Fig. 2), obtained in optically pure forms by diastereoisomeric differential crystallization with suitable acids or by derivatization with chiral α-methylbenzylamine, we envisaged that kinetic resolution of 1-methyl-3-phenylpropylamine, (±)-**3** via enzymatic benzoylation could give an alternative access to the key intermediate **2a** without the need of any chiral

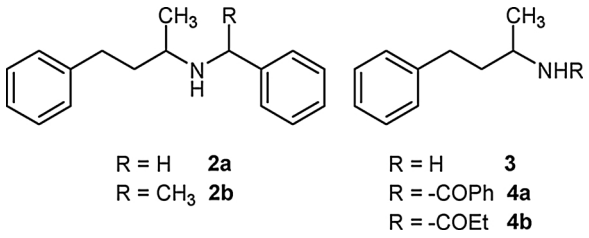

Fig. 2. Synthetic precursors of labetalol.

In this context, the feasibility of a lipase-catalyzed benzoylation was checked on some selected amines and herein we report the obtained results. The optimization of the process for kinetic resolution of amine (±)-**3** allowed us to obtain benzamide (*R*)-**4a** as a precursor of (*R*)-**2a**, that was then chemically converted into the 1:1 mixture of pharmacologically active *R,R*- and *S,R*-isomers of labetalol.

2. Experimental

2.1. Materials

5-(2-Bromoacetyl)-2-hydroxybenzamide was purchased from Alfa Aesar, (±)-α-methylbenzylamine from Acros Organics and the other chemicals from Aldrich. Lipases from *Candida antarctica* B immobilized on acrylic resin (Novozym 435) and from *Mucor miehei* immobilized on macroporous ion-exchange resin (Lipozyme) were obtained from Sigma. Amano Lipase PS-C II (immobilized on ceramic) and lipase A from *Candida antarctica* cross-linked aggregate (CAL-A) were purchased from Fluka. Solvents for enzymatic reactions were dried over activated molecular sieves (3 Å, Aldrich) prior the use. Thin layer chromatography (TLC) was carried out on Merck silica gel 60-F254 precoated glass plates.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance™ 400 instrument at 400.13 and 100.03 MHz respectively. Chemical shifts (δ) are reported in ppm relative to TMS and coupling constants (*J*) are in Hz.

The enantiomeric excesses of chiral compounds were determined by HPLC using Lux Cellulose columns (250 × 4.60 mm, Phenomenex) isocratically eluted at 23 °C with *n*-hexane/2-propanol mixtures at 0.5 mL/min flow rate and simultaneous detection at 220, 250, 266 and 275 nm. Optical rotations were recorded on a DIP 135 JASCO instrument using a f 3.5′ 100 mm cell.

High resolution mass spectra (HR-MS) were acquired by a Thermo Scientific Exactive Plu Orbitrap MS (Thermo Fisher Scientific, Inc., Milan, Italy) instrument with ESI ionization mode using a Thermofisher Orbitrap QExactive instrument, set with 3.0 kV source voltage and 300 °C capillary temperature.

2.2. General procedure for aminolysis of methyl benzoate catalyzed by Novozym 435

To an equimolecular (0.62 mmol) solution of amine substrate (see Table 1) and methyl benzoate in 1 mL of anhydrous toluene, Novozym 435 (50 mg) and molecular sieves (40 mg) were added. The suspension was then stirred in a shaker at 300 rpm at 45 °C. The reaction was monitored by TLC analysis (*n*-hexane/EtOAc 70:30) and quenched at suitable time by filtering off the solids. The solution was taken to dryness and an aliquot of the residue analysed by ^{1}H NMR for the determination of substrate conversion, by means of integration of the resonances for C*H*-NH_2 and C*H*-NHCOPh protons. The residue was then partitioned between EtOAc and aqueous HCl to give unreacted amine in the aqueous phase. The organic phase was washed with $NaHCO_3$, dried over Na_2SO_4 and taken to dryness to give pure benzamides **4a** and **8**-**10**. The NMR data of all the obtained benzamides were in agreement with reported data [16,22,23].

The enantiomeric excesses of **4a**, **9** and **10** were measured by chiral HPLC chiral analyses on Lux Cellulose-1 column: **4a** (*n*-hexane/2-PrOH 85:15) t_R/min: 24.21 [(*R*)-**4a**] and 35.59 [(*S*)-**4a**]; **9** (*n*-hexane/2-PrOH 90:10) t_R/min: 32.09 [(*R*)-**9**] and 42.92 [(*S*)-**9**], **10** (*n*-hexane/2-PrOH 85:15) t_R/min: 17.45 [(*S*)-**10**] and 22.49 [(*R*)-**10**].

The absolute configuration of (*R*)-**9** was assigned by comparison of the HPLC retention time of the product of enzymatic reaction with an authentic sample obtained by chemical benzoylation of commercially available (*R*)-**6** and confirmed by its optical rotatory power $[\alpha]_D^{25} = +19.7$ (*c* 1.0, $CHCl_3$), lit. $[\alpha]_D^{19} = -20.1$ (*c* 1.0, $CHCl_3$) [24]. The *R*-configuration was also assigned to the enzymatically obtained **4a** on the basis of its optical rotation, $[\alpha]_D^{25} = -6.3$ (c 0.9, CH_2Cl_2), lit. $[\alpha]_D^{25} = +6.0$ (c 0.25, CH_2Cl_2) for the (*S*)-**4a**, [22].

2.3. General procedure for lipase-catalyzed kinetic resolution of (±)-3

To a solution of (±)-**3** (100 µL, 0.62 mmol) and methyl benzoate (78 µL, 0.62 mmol) in 1 mL of dry toluene 50 mg of the lipase of choice and 40 mg of molecular sieves were added. The resulting heterogeneous mixture was shaken at 300 rpm and 45 °C. The reaction progress was monitored by TLC chromatography (*n*-hexane/EtOAc 70:30) and at