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Milnacipran as a challenging example of aminomethyl substrate for lipase-catalyzed kinetic resolution

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ABSTRACT

A biocatalysed procedure for the kinetic resolution of milnacipran, (±)-1 was developed and optimized by careful choice of the reaction parameters. The reaction of (±)-1 with methyl *iso*-butyrate as acyl donor in the presence of Novozyme 435 in *tert*-butyl methyl ether proceeded with moderate enantioselectivity giving the more pharmacologically active enantiomer of milnacipran (−)-1 as unreacted substrate and the corresponding amide (−)-4 both in optically enriched form. When the enzymatic reaction was prolonged up to 65% substrate conversion enantiopure levomilnacipran (−)-1 (98% *ee*) was directly recovered from the reaction mixture by simple extraction workup.

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1. Introduction

Chiral amines are important building blocks and final products in pharmaceutical and agrochemical industries and also find useful application in organic chemistry as chiral auxiliaries, ligands and resolving agents [1–3]. Their preparation in enantiopure form relies on resolution methods [4–7], mainly based on the differential crystallization of their diastereoisomeric salts with chiral acids [8,9], or asymmetric synthesis [10–12] by means of an astonishing variety of reactions promoted by chiral metal-ligand complexes or organocatalysts.

Biocatalysed methods offer valuable alternatives for the availability of different enzymes, as hydrolases, transaminases or amine oxidases, able to perform kinetic resolution of racemic amines in aqueous medium or organic solvent [13,14]. In this context, lipases have gained increased popularity since they do not require cofactors, can be reused when immobilized on suitable supports and, most important, are compatible with a variety of racemization chemocatalyst so allowing the preparation of enantiopure amines in up to 100% theoretical yield through a dynamic kinetic resolution (DKR) process [15].

The lipase-catalysed reaction of a racemic amine in the presence of a suitable acyl donor in organic solvent leads to the irreversible and enantioselective *N*-acylation of substrate, since in these conditions amide bonds are unreactive towards the enzyme. Most investigation in this field have focused on the kinetic resolution of (±)-1-phenylethylamine [16–19] and related α-branched primary amines [20,21] as well as on the development of effective racemization catalysts for DKR [22], whereas secondary amines have been less considered [23].

Other applications are the direct reaction of carboxylic acids (or esters) with achiral primary amines as a benign and mild method for the synthesis of *N*-substituted amides [24–26] and the kinetic resolution of chiral acids *via* aminolysis of the corresponding esters [27–29].

In analogy with chiral primary alcohols, whose lipase-catalysed transesterification can sometimes proceed with low enantioselectivity related with the high reactivity of hydroxyl group and the distance between the stereogenic and reactive centres [30–32], chiral amines containing an aminomethyl group are expected to be challenging substrates and, at the best of our knowledge, their biocatalytic resolution is limited to few cases [33,34].

With the aim to expand the potentiality of lipase-catalysed enantioselective reactions, we planned to investigate the biocatalytic resolution of milnacipran, (1*R**,2*S**)-2-(aminomethyl)-*N*,*N*-diethyl-1-phenylcyclopropanecarboxamide, (±)-**1**], chosen as an example of primary amine bearing remote stereogenic centre(s). Compound **1**, originally developed as an antidepressive drug belonging to the class of selective serotonin reuptake inhibitors [35], has recently attracted renewed interest for its positive effects in relieving the chronic pain associated with fibromyalgia [36]

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and it is marketed as racemic hydrochloride salt (Ixel®, Savella®) (Scheme 1).

Pharmacokinetic studies on the separate enantiomers of **1** showed higher activity for levomilnacipran, (1*S*, 2*R*)-(−)-**1** [37,38] and, in order to meet FDA and EMA guidelines, there is a growing interest in the preparation of enantioenriched (−)-**1** for novel drug formulations [39,40]. Although some synthetic methodologies for the preparation of optically active **1** have been reported [41,42], the availability of a biocatalytic protocol for kinetic resolution of (±)-**1** could be valuable in terms of costs and operational simplicity. Here we report the obtained results in lipase-catalysed *N*-acylation of (±)-**1** and the preparation of both enantiomers of the drug in optically active form.

2. Experimental

2.1. General information

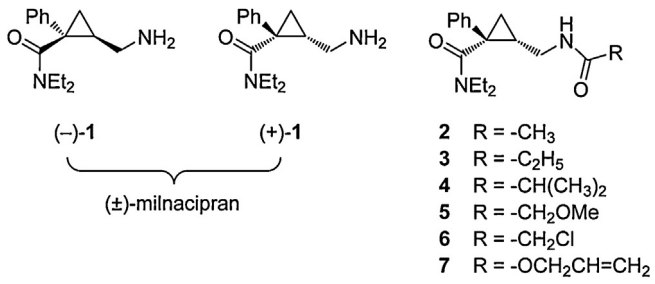
Immobilized lipases PS-C I (from *Pseudomonas cepacia*), Novozyme 435® (from *Candida antarctica*, CAL-B) and crude Lipase AK (from *Pseudomonas fluorescens*) were purchased from Aldrich, Lipozyme® (immobilized lipase from *Mucor miehei*) was obtained from Fluka, PPL (crude lipase from *Porcine pancreas*) and cross-linked enzyme aggregate CLEA (from *Candida antarctica*) were from Sigma, Chirazyme L-9 (immobilized lipase from *Rhizomucor miehei*) was purchased from Boehringer Mannheim. Analytical grade chemicals were used as received. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker Avance™ 400 instrument at 400.13 and 100.03 MHz, respectively. Chemicals shifts (δ) are reported in ppm relative to the residual solvent and coupling constants (J) are given in Hz. Optical rotations were recorded on a DIP 370 JASCO instrument using a ϕ 3.5 × 100 mm cell.

2.2. Extraction of (±)-*1* from the pharmaceutical preparation

Milnacipran free base (±)-**1**, was recovered from commercial pharmaceutical preparation according the following procedure: the tablets were grinded in a mortar and suspended in methanol. The suspension was stirred at room temperature for 1 h, then filtered and the solution evaporated to dryness. The residue was suspended in aq. 2 N NaOH and the solution stirred for 30 min at room temperature, then the aqueous phase was extracted with *tert*-butyl methyl ether (*t*-BME). The organic solution was dried on Na_2SO_4 and taken to dryness to give (±)-**1** as a pale yellow oil, with analytical purity 98% and spectral properties in agreement with literature data [41,42].

2.3. Chiral HPLC analyses

The enantiomeric excesses were determined by chiral HPLC at 23 °C on Phenomenex Lux® Cellulose-4 (250 × 4.60 mm) column using isocratic elution with *n*-hexane/2-propanol 70:30 (v/v), flow rate 0.5 ml/min and UV-detection at λ 220 nm. Pre-column derivatization with propionic anhydride was applied to all the samples from the enzymatic reactions, with the exception of samples from enzymatic reaction with ethyl propionate as acyl donor that were treated with acetic anhydride. Reference samples of racemic amides (±)-**2**–(±)-**5** were obtained by reaction of (±)-**1** with the suitable anhydride while (±)-**7** was obtained by reaction of (±)-**1** with allyl chloroformate in CH_2Cl_2 in the presence of pyridine.



Scheme 1. Chemical structures of milnacipran enantiomers and related amide derivatives.

Retention times of the racemic reference compounds: *N*-acetyl milnacipran (±)-**2**: t_R 21.2 min (1*S*,2*R*)-**2** and 37.5 min (1*R*,2*S*)-**2**; *N*-propanoyl milnacipran (±)-**3**: t_R 16.6 min (1*S*,2*R*)-**3** and 23.0 min (1*R*,2*S*)-**3**; *N*-*iso*-butanoyl milnacipran (±)-**4**: t_R 12.1 min (1*S*,2*R*)-**4** and 14.2 min (1*R*,2*S*)-**4**; *N*-(2-methoxy)-ethanoyl milnacipran (±)-**5**: t_R 31.4 min (unresolved); *N*-allylcarbamoyl milnacipran (±)-**7**: t_R 15.1 min (1*S*,2*R*)-**7** and 21.9 min (1*R*,2*S*)-**7**. Enantiomeric excesses for products (ee_p) and milnacipran (ee_s), the latter converted into a suitable amide derivative, were determined from peak areas of the corresponding enantiomers following $ee = (A_> - A_<)/(A_> + A_<) \times 100$. Conversion ($C$) values in kinetic resolution experiments were then derived from $C = ee_s/(ee_s + ee_p)$. Since amide **5** eluted as a single peak, the conversion value of entry 4 in Table 1 was obtained by direct comparison of peak areas after correction of area of **5** with respect to **3** by a relative response factor RRF = 1.90.

2.4. General procedure for the lipase-catalyzed resolution of (±)-*1*

In a general enzymatic reaction, 10 mg (0.04 mmol) of (±)-**1** were dissolved in 2 ml of solvent, then 30 mg of lipase and acyl donor (0.43 mmol) were added and the resulting suspension was shaken at 300 rpm and 40 °C. Aliquots were withdrawn at various intervals and subjected to chiral HPLC analysis to determine both conversions and enantiomeric excesses. In the experiment in presence of molecular sieves, they were added (40 mg) together with the lipase to the reaction mixture. The same experimental conditions were also applied to enzymatic reactions at variable temperature.

2.5. Semi-preparative resolution of (±)-*1* by lipase catalyzed reaction

In a semi-preparative run (±)-**1** (200 mg, 0.81 mmol) was dissolved in a mixture of *t*-BME and methyl *iso*-butyrate (M*i*Bu) as acylating agent in 3:2 v/v ratio (20 ml) and the reaction was started by adding Novozyme 435 lipase (600 mg). The suspension was shaken at 300 rpm and 50 °C until the HPLC analysis showed about 50% of substrate conversion (1.5 h). The enzyme was then filtered off and the solution evaporated to dryness. The residue was dissolved in 1 N HCl (5 ml) and the solution extracted with *t*-BME. The organic phase was dried on anhydrous Na_2SO_4 and evaporated under *vacuum* to give the amide product (−)-**4** as a white solid in 51% yield (128 mg, 0.41 mmol) and 71% *ee*.

The acidic aqueous phase was alkalinized by addition of 2 N NaOH and then extracted with *t*-BME. The organic phase was dried on anhydrous Na_2SO_4 and taken to dryness to afford unreacted (−)-**1** as yellow pale oil in 41% yield (81 mg, 0.33 mmol) and 76% *ee*.

Crystallization of (−)-**4** from *t*-BME gave white crystals in enantiopure form (>98% *ee*) and 68% crystallization yield (88 mg, 0.28 mmol). Data for (−)-**4**: $[\alpha]_D = -148.9$ (c 0.67, $CHCl_3$), mp 138 °C; ^{1}H NMR: δ 0.84 (3*H*, t, J = 7.1 Hz, CH_3CH_2-), 1.13 (10*H*, m, CH_3CH-, CH_3CH_2- and $-CH-$), 1.45 (1*H*, m, $-CHCH_2NH-$), 1.54 (1*H*, dd, J = 8.8 and 5.2 Hz, $-CH-$), 2.39 (1*H*, heptet, J = 6.8 Hz, CH_3CH-), 2.59 (1*H*, m, $-CH_2NH-$), 3.33 (2*H*, m, CH_3CH_2-), 3.41 (1*H*, m, CH_3CH_2-), 3.49 (1*H*, m, CH_3CH_2-), 4.10 (1H, m, $-CH_2NH-$), 7.18 (3*H*, m, Ph), 7.27 (2*H*, m, Ph); ^{13}C NMR: δ 12.4, 13.0, 17.4, 19.5, 19.7, 26.9,

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