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One-pot biocatalyzed preparation of substituted amides as intermediates of pharmaceuticals

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Abstract

A lipase-catalyzed procedure is described for the one-pot conversion of carboxylic acids into substituted amides via in-situ formation of the ethyl ester and subsequent aminolysis. The procedure was optimized for the preparation of tetrahydro-*N*-[3-(methylamino)-propyl]-2-furancarboxamide, an intermediate in the synthesis of Alfuzosin, a reducing agent of symptoms associated with benign prostatic hypertrophy. This methodology proved to be general and can be applied to open-chain, cyclic, hydroxy-, amino-, dicarboxylic, various chain lengths, and unsaturated acids. Moreover, the enzyme shows a regioselective behavior in relation to primary and secondary amino groups. The procedure involved the treatment of the corresponding carboxylic acid with ethyl alcohol in presence of immobilized *Candida antarctica* lipase followed by addition of amine. The amide is obtained in good yields and regioselective way. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Lipase; Substituted amides; One-pot synthesis; Alfuzosin; Pharmaceutical intermediates

1. Introduction

Biological tools are applied in synthetic processes for the preparation of food additives, pharmaceuticals, and fine and commodity chemicals [1,2]. The discovery that some enzymes such as lipases, catalyze esterification and transesterification reactions in organic solvents [3], allowed the preparation of compounds, which are difficult to obtain by chemical methods. Considering this approach, we have performed biocatalyzed acylation and desacylation reactions on various substrates such as hydroxyalkanethiols [4–10], steroids [11,12], vitamin B_6 [13], etc.

The use of amides as protection for a group of amino compounds, and their extensive occurrence in bio-active compounds [14] make their formation an important task in organic synthetic chemistry.

The application of lipases to catalyze amide bond formation is an interesting alternative to conventional methods that use proteases because lipases can act in low hydrated organic solvents [15] showing nule or very low amidase activity. Amidation reactions catalyzed by lipases have been applied to the synthesis of peptides from protected D- or L-

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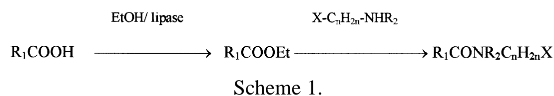


Scheme 1.

aminoacids [16,17], preparation of aminosugar derivatives [18], aminolysis of esters in the resolution of chiral carboxylic acids and chiral amines [19,20], etc. Lipase-catalyzed ammoniolysis reactions, where ammonia serves as the nuclophile, were successfully accomplished with esters [21–23] and aminoacid esters [23,24] and in the preparation of carboxylic amides from carboxylic acids [25]. Here, we report an easy one-pot procedure for the lipase-catalyzed preparation of substituted amides from carboxylic acids according to Scheme 1.

Moreover, we have applied this one-pot procedure to the synthesis of an intermediate of alfuzosin (Fig. 1).

Alfuzosin, a quinazoline derivative, acts as a potent, competitive, and selective antagonist of α_1-adrenoreceptor-mediated contraction of prostate, prostatic capsule, proximal urethral and bladder base smooth muscle, thereby reducing symptons associated with benign prostatic hypertrophy [26,27]. Benign prostatic hypertrophy is considered to be a major health hazard in modern society. The prevalence of histological benign hyperplasia increases from approximately 8% in the fourth decade of life to approximately 90–100% in the ninth decade [28].

Several routes have been reported for the chemical synthesis of alfuzosin, with tetrahydro-*N*-[3-(methylamino)-propyl]-2-furancarboxamide **12b** as the most widely used intermediate [29]. Its preparation from 2-tetrahydrofuroic acid is difficult, involving toxic and air sensitive reagents and drastic reaction conditions [30]. We report that through enzymatic methodology, it is possible to obtain compound **12b** in a one-pot procedure, in very good yield and mild reaction conditions (Scheme 2).

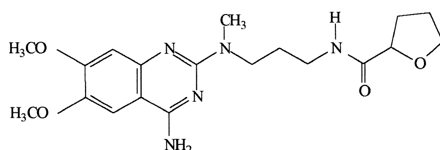
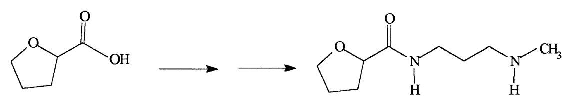


Fig. 1.



Scheme 2.

2. Experimental

2.1. Analysis and materials

The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ and $CDCl_3$/MeOD with TMS as internal standard using a Varian Brucker AC-200 spectrometer. Analytical Gas Chromatography (GC) was performed using a Hewlett Packard 5890 gas chromatograph with a Carbowax 20H-022 capillary column (50 m × 0.32 mm). For TLC, Merck silica gel 60F-254 aluminum sheets (0.2 mm thickness) were used. Gas Chromatography-Mass Spectrometry (GC-MS) spectra were performed using a gas chromatograph coupled to a Varian Mat CH7-A spectrometer interfaced to a Varian-Mat Data System 166 and on a VG-TRIO-2-GC-MS instrument.

All solvents and reagents were of analytical grade. Lipase from *Candida cylindracea* or *rugosa* (905 units/mg solid), lipase Type II crude from porcine pancreas (190 units/mg protein) were purchased from Sigma. Lipozyme, lipase IM-60 from *Mucor miehei* in the immobilized form on a microporous anion exchange resin and *C. antarctica* lipase: Novozym 435 (7400 PLU/g) acrylic resin-supported lipase produced by a host organism, *Aspergillus oryzae*, after the transfer of the genetic coding for lipase B from *C. antarctica*, were generous gifts of Novo Nordisk Bioindustrial Group. Papain (2648 units/mg solid) and chymotrypsin (1311 units/mg solid) were a gift of Biobras. Fungal protease (31000 HU/g) and bacterial protease (200 NU/g) were provided by Solvay Enzimas. All enzymes were used "straight from the bottle".

2.2. General one-pot procedure

Lipase Novozym 435 (500 mg) was added to a solution of the carboxylic acid (3 mmol) in absolute ethanol (5 ml). The suspension was shaken (200 rpm) at 30°C and the progress of the reaction was monitored by GC or TLC. When acid was converted into the ethyl ester, amine (4 mmol) was added. After the indicated time, the enzyme was filtered off, then the solvent was evaporated, and the crude residue purified by flash chromatography on silica gel and identified by GC-MS and by ^{1}H and ^{13}C NMR spectroscopies. Tests with proteases were performed with 3 mmol of carboxylic acid and 4 mmol of *n*-propylamine (200 rpm, 30°C), with acetonitrile as solvent.

Table 1
Enzyme-catalyzed one-pot esterification and aminolysis of hexanoic acid[a]

Lipase	% Conversion[b]	
	Ester	Amide
None	n.d.	n.d.
Porcine pancreatic lipase	96	68
Lipozyme	97	64
Candida rugosa lipase	98	78
Candida antarctica lipase (CAL)	100	100
Chymotrypsin[c]	–	2
Papain[c]	–	n.d.
Bacterial protease[c]	–	n.d.
Fungal protease[c]	–	n.d.

[a] Reactions were performed under standard conditions; time: ester conversion: 3 h; amide conversion: 3 days.
[b] Determined by GC and GC/MS analysis; n.d.: not detected.
[c] Reaction of acid with amine.

3. Results and discussion

3.1. Optimization of the reaction conditions

Four commercial lipases and four proteases were tested in the esterification and aminolysis of hexanoic acid. Lipases included porcine pancreas lipase, lipozyme, immobilized *Mucor miehei* lipase, *C. rugosa* lipase, and immobilized *C. antarctica* lipase B (CAL). Proteases included papain, chymotrypsin, bacterial protease, and fungal protease. As it can be seen from Table 1, none of the proteases-afforded product and lipases showed variable activity (CAL giving the most satisfactory results).

Enzymatic esterification was tested with absolute ethanol, isopropanol, and *n*-butanol. They acted both as solvent and esterification agent. The three alcohols afforded the corresponding ester in quantitative yield after 3 h of reaction. Another test was performed using commercial ethanol 96% but, as ester was not obtained in quantitative yield, amide yield decreased to 23%.

The enzymatic reaction of hexanoic acid with *n*-propylamine was carried out at different acid and amine concentrations. The best yield was obtained at an amine/acid molar ratio equal of 1.3 and at 0.6 M acid concentration. The amount of CAL in the reaction system was varied from 10 to 1000 mg at 0.6 M acid concentration and at 1.3 amine/acid molar ratio. Yields of 20% of amide were observed by just using 100 mg of lipase, with 500 mg giving the best results (100%). As regards the possibility of enzyme re-use, we observed a loss of only 10% in yield when the recovered enzyme was used in eight consecutive reactions with fresh substrate, ethanol, and amine.

3.2. Application of the enzymatic one-pot procedure to several carboxylic acids

The results obtained by means of the enzymatic one-pot procedure to the preparation of substituted amides from several carboxylic acids are shown in Table 2. Variable chain length carboxylic acids from 3 to 18 carbon atoms gave the ethyl esters in quantitative yield and mild condition reaction in a few hours (compounds **1a**–**9a**). Ester formation was not influenced by chain length. Increasing the temperature reaction from 30°C to 40°C (**5**, **6**, **7**) diminished the time reaction both in esterification and aminolysis steps but caused a slight decrease in amide yield.

In the case of unsaturated acids, both *N*-propyloleamide **8b** and *N*-propylelaimide **9b** were obtained in high yield under these mild conditions. Due to *N*-alkyalmides have insect-repellent properties, several efforts have been made to prepare them by

Table 2
Lipase-catalyzed preparation of amides from carboxylic acids[a]

Compound	R_1	*n*	R_2	X	Ester **a**		Amide **b**	
					t (h)	%	*t* (day)	%
1	CH_3CH_2-	2	H	CH_3	2	100	2	93
2	$CH_3(CH_2)_4-$	2	H	CH_3	3	100	3	100
3	$CH_3(CH_2)_4-$	2	H	OH	3	100	3	100
4	$CH_3(CH_2)_4-$	3	H	$NHCH_3$	3	100	3	100
5	$CH_3(CH_2)_8-$	2	H	CH_3	3	100	7	98
5	$CH_3(CH_2)_8-$[b]	2	H	CH_3	1	100	5	90
6	$CH_3(CH_2)_{12}-$	2	H	CH_3	3	100	8	85
6	$CH_3(CH_2)_{12}-$[b]	2	H	CH_3	1	100	4	79
7	$CH_3(CH_2)_{16}-$	2	H	CH_3	3	100	8	87
7	$CH_3(CH_2)_{16}-$[b]	2	H	CH_3	1	100	4	75
8	$CH_3(CH_2)_7CH{=}CH(CH_2)_7-$ *cis*	2	H	CH_3	3	100	8	89
9	$CH_3(CH_2)_7CH{=}CH(CH_2)_7-$ *trans*	2	H	CH_3	3	100	8	86
10	cyclopentyl–	3	H	$NHCH_3$	3	100	2	76
11	tetrahydrofuryl–	3	CH_3	CN	3	100	2	81
12	tetrahydrofuryl–	3	H	$NHCH_3$	3	100	1	72
13	H_2NCH_2-	2	H	CH_3	168	n.d.	15	n.d.
14	$H_2N(CH_2)_3CH(NH_2)-$	2	H	CH_3	168	n.d.	15	n.d.
15	$CH_3CH(NH_2)CH_2-$	2	H	CH_3	168	n.d.	15	n.d.
16	$H_2N(CH_2)_3-$	2	H	CH_3	168	n.d.	15	n.d.
17	$H_2N(CH_2)_5-$	2	H	CH_3	168	46	15	n.d.
18	*p*-$H_2NC_6H_4-$	2	H	CH_3	168	n.d.	15	n.d.
19	$HOOC(CH_2)_2-$	2	H	CH_3	24	100[c]	2	73[d]
20	$HOOC(CH_2)_2-$	2	H	CH_3	–	–	6	57[e]
21	$CH_3CH(OH)-$	2	H	CH_3	24	100	4	100
22	$HOOCCH(OH)CH(OH)-$	2	H	CH_3	168	n.d.	15	n.d.
23	$HOOCCH_2C(OH)(COOH)CH_2-$	2	H	CH_3	168	n.d.	15	n.d.

Reactions were performed under standard conditions unless indicated. n.d.: not detected.
[a] % Conversion determined by GC.
[b] 40°C.
[c] Diethyldiester.
[d] Amidoester.
[e] Succinimide.

chemical methods. Some of them need drastic conditions of pressure and temperature [31], air-sensitive reagents [32], or several steps involving mono-*N*-alkylation of amides via the reduction of methylols derivatives with trialkylaluminum reagents [33].

In order to investigate the effect of the nature of the amine on the aminolysis reaction, ethanolamine and *N*-methyl-1,3-propanediamine were tested with hexanoic acid. *C. antarctica* lipase acted chemoselectively, giving the ethylolamide **3b** as the only product in the first case while with the diamine afforded product **4b** in a regioselective way. Enzyme exhibited a remarkable preference for the primary amino group over the secondary one. Both products **3b** and **4b** were obtained in quantitative yields. This regioselective lipase behavior was also observed when the procedure was applied to the preparation of cyclopentyl carboxamide **10b**.

Succinic acid was used as model of diacid substrate. Diester **19a** was obtained in quantitative yield but aminolysis gave different products depending on reaction time. After 2 days of reaction with *N*-propylamine, lipase catalyzed the selective monoaminolysis of diethyl succinate giving amidoester **19b** in good yield. At longer reaction times such as 6 days, a mixture of the *N*-propylsuccinimide **20b** and ami-

doester **19b** was obtained. The formation of *N*-propylsuccinimide probably takes place by heterocyclization of the amidoester **19b**, with a nucleophilic role of the amide nitrogen. This result is in accordance with a previously reported outcome [34].

Lactic acid, a hydroxyacid, behaves as an excellent substrate both in esterification and aminolysis reactions, producing hydroxyamide **21b**. More functionalized acids, such as tartaric and citric acids, failed to give the corresponding esters **22a** and **23a** in the first step, perhaps due to steric hindrance, which could preclude the interaction with the enzymatic active site.

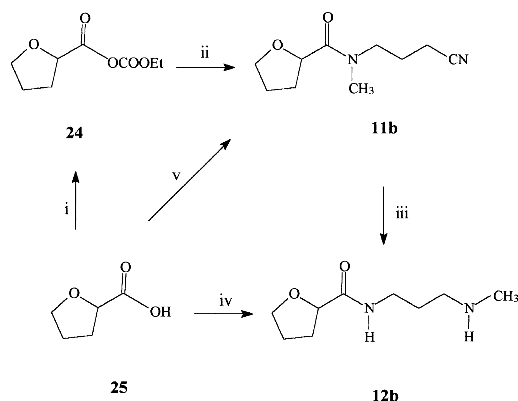
Then, we tested aminoacids as substrates: glycine, ornithine, β- and γ-aminobutyric acids, 6-aminohexanoic acid and *p*-aminobenzoic acid. With the exception of 6-aminohexanoic acid, which gave the ester with poor results (**17a**), it was not possible to obtain the corresponding amides (**13b–18b**) by using the present procedure.

3.3. Application of the enzymatic one-pot procedure to the preparation of a pharmaceutical intermediate

The one-pot procedure was applied to the preparation of **12b**, intermediate in the synthesis of alfuzosin, as mentioned in Section 1.

Previous work [29,30] describing the chemical synthesis of **12b** and related compounds reported that they were prepared by treatment of carboxylic acid derivatives with aminonitriles to give nitrileamides intermediates of type **11b**, which by reduction produced compounds of type **12b** as described in Scheme 3.

Synthesis used a mixed anhydride **24** as functional derivative of the carboxylic acid, prepared by treatment of the carboxylic acid **25** with ethyl chloroformiate and triethylamine at low temperatures and under nitrogen atmosphere. Compound **11b**, product reaction of **24** with 3-(methylamino)-propanenitrile was to be distilled at reduced pressure. This nitrile was hydrogenated in an ethanolic ammonia solution at 80°C over Rh/C at 840 psi hydrogen pressure, affording **12b** through a rearrangement involving the migration of methyl group from one nitrogen to the other.



Scheme 3.

In the procedure described in the present work, product **12b** is obtained by addition of *N*-methyl-1,3-propanediamine to the reaction vessel where tetrahydrofurancarboxylic acid ethyl ester has been easily formed by lipase-catalyzed reaction of the acid **25** with ethanol.

All the procedure is carried out in one pot without the isolation of tetrahydrofurancarboxylic acid ethyl ester, which is obtained in quantitative yield through enzymatic catalysis. Ethanol acts both as reagent and solvent. CAL's good performance is remarkable in excess of ethanol, a reagent which is not considered an appropriate solvent for enzymatic reactions carried out in organic medium.

Considering the reported chemical synthesis, a preliminary test of enzyme-catalyzed reaction of the tetrahydrofuroic acid ethyl ester with 3-(methylamino)-propanenitrile to obtain compound **11b**, was accomplished with very good results (81%) (See Scheme 3). But this product **11b** needs an additional step in the synthetic way driving to **12b**.

Enzymatic catalysis allowed the reaction at near room temperatures (30°C). As enzyme is insoluble in the reaction media, it is easily removed by filtration at the end of the process. It can be re-used and in this particular reaction, CAL keeps 80% activity after eight reaction cycles. CAL shows high regioselectivity in the aminolysis of ester, producing exclusively the less-substituted amide.

We carried out a preparative scale reaction in the same way as described above with 20 g of CAL. Even though the yield of **12b** showed a slight de-

crease from 72% to 69%, the result confirmed the feasibility of the procedure for large-scale production of the intermediate **12b** [36].

Moreover, the application of enzymatic approach to the preparation of **12b** is environmentally acceptable. First of all, biocatalysts are completely degraded in the environment. Simple and mild reaction conditions are remarkable. Neither high toxic and water sensitive reagents, such as ethyl chloroformiate, are necessary, nor high-pressure hydrogenation with expensive catalysts. On the other hand, the global yield of product **12b** from tetrehydrofuroic acid is 72% (Table 2) better than the one obtained by chemical synthesis, which reached 41% at best [35]. Purification of **12b** is easy due to the high selectivity of enzymatic catalysis with no secondary reaction products.

4. Conclusion

An efficient one-pot procedure for the enzymatic preparation of *N*-substituted carboxamides from the corresponding carboxylic acids via the formation of carboxylic ethyl esters has been developed. This one-pot procedure provides a simple and mild alternative method for the synthesis of substituted amides proceeding from a variety of carboxylic acids: long open chain, cyclic, hydroxy, dicarboxylic unsaturated, etc. Among them is the remarkable preparation of tetrahydro-*N*-[3-methylamino-propyl]-2-furancarboxamide, intermediate in the synthesis of alfuzosin. The reactions use a lipase as catalyst and require low-cost and available raw materials.

Acknowledgements

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