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LIPASE-CATALYSED ADDITION OF PYRROLIDINE TO CHALCONE AND BENZYLIDENE MALONATE DERIVATIVES

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The porcine pancreas lipase (PPL) type II catalyzes the aminolysis of benzylidene malonates by regiospecific amidation of substrates and afforded the Z*-isomer; no* E*-isomer or diamide byproducts were observed. PPL also catalyzes Michael addition of acetophenone to various derivatives of chalcones.*

Keywords: benzylidene malonates, chalcones, regioselectivity, Michael addition, porcine pancreas lipase.

Lipases (E.C. 3.1.1.3), in addition to their natural function of hydrolyzing the carboxylic acid esters, can catalyze hydrolysis, esterification, and transesterification, including alcoholysis and amidation. Lipases are the most widely used enzymes in organic synthesis [1–5]. Some lipases can catalyze different reactions from the natural process in nonconventional organic synthesis reactions such as carbon-carbon bond formation and carbon-heteroatom and heteroatom-heteroatom bond formations [6]. Michael addition reactions are traditionally catalyzed under strong basic or acidic conditions, which can cause side reactions with the formation of undesired products such as polymerization of the Michael acceptor or cyclocondensations [7–9].

During our investigations devoted to finding new synthetic applications of lipases as biocatalysts, porcine pancreas lipase (PPL) activity was evaluated in Michael-type addition reactions of secondary amines to α,β-unsaturated compounds. In this regard the reaction of pyrrolidine as a good nucleophile with various chalcone and benzylidene malonate derivatives as Michael acceptor was investigated. To the best of our knowledge, this is the first report of PPL catalytic activity in these reactions.

Aza-Michael additions represent 1,4-addition of a nitrogene nucleophile to an α,β-unsaturated compound. In the hydrolases family, enzymes such as lipases can catalyze this type of reaction. In continuation of our ongoing research on lipase-catalyzed organic reactions, we decided to examine the ability of PPL in *aza*-Michael addition of amines to α,β-unsaturated compounds. By considering the reports, we envisioned that PPL can catalyze this type of reaction [10, 11]. The reaction of benzylidene malonate derivatives with different secondary amines (diethylamine, pyrrolidine, and piperidine) was investigated, but instead of the *aza*-Michael addition, regiospecific aminolysis was observed.

Among the three secondary amines, pyrrolidine showed the best results regarding yield and reaction rate, so further examinations were performed on pyrrolidine.

Three different conditions were designed in order to determine if the reaction proceeds spontaneously or is catalyzed by enzyme: 1) reaction without enzyme at room temperature; 2) reaction without enzyme by reflux in toluene at 110°C; 3) reaction in the presence of enzyme at room temperature. It was observed that the product was obtained only in the enzyme-catalyzed reaction at room temperature after 96 h. ^{1}H NMR spectra of the product indicated that one of the carboxylic acid ester groups reacted with amine and that was the only product. The overaminated product and the *E*-isomer were not detected in the ^{1}H NMR spectra. The structures of the products were determined as the *Z*-isomer by NOESY. A NOESY relationship was observed between the aromatic proton and the methylene protons (H_3CCH_2–O–).

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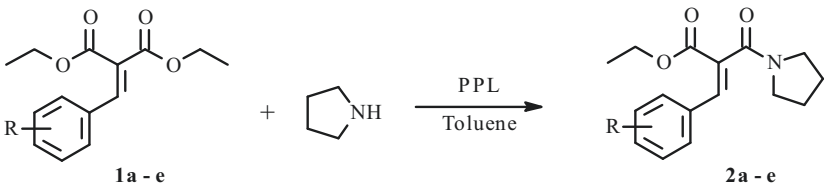
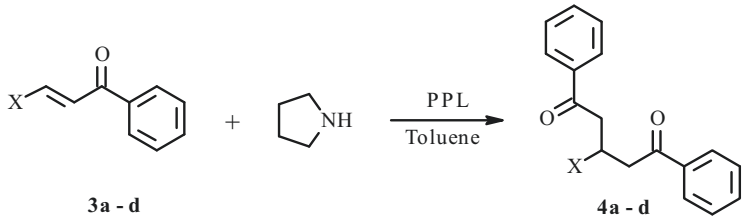


1a - e **2a - e**

R = H (**a**); *p*-Cl (**b**); *p*-NO_2 (**c**); *p*-$N(CH_3)_2$ (**d**); *o*-OCH_3 (**e**)



3a - d **4a - d**

X = *p*-ClC_6H_4 (**a**); *p*-FC_6H_4 (**b**); *p*-CH_3O-C_6H_4 (**c**); 2-naphtyl (**d**)

Chloro- and nitro groups (entries 2, 3) are representative of electron-withdrawing groups; *N*,*N*-dimethyl and methoxy (entries 4, 5) are representative of electron-donating group. All the substrates reacted to afford the *Z*-aminated product (**2a**–**2e**) with complete regioselectivity. Substrates with electron-withdrawing groups gave relatively higher yields compared to other substrates. Entry 5 containing the methoxy group at the *ortho*-position, probably due to greater hindrance, were poor substrates for aminolysis. There are some reports about regioselective acetylation of 1,3-diols catalyzed by lipase. Miura and co-workers reported regiospecific acetylation of 2-alkylidene propane-1,3-diols, yielding (*E*)-2-(hydroxymethyl)prop-2-enyl acetates as the sole product [12].

The second α,β-unsaturated compounds that were selected to investigate the lipase catalytic ability in nonconventional enzymatic reactions were chalcone derivatives. Different substrates were synthesized according to the method mentioned above and reacted with pyrrolidine as nucleophile.

In order to activate the carbonyl group, chalcones and the enzyme were stirred on an orbital shaker (120 rpm, r.t) for 1 h; then an excess of pyrrolidine was added and the reaction was monitored by TLC. After one day, one product was observed by TLC. At the end of the fourth day, only one product was produced. The products were purified. In the ^{1}H NMR spectra, the signals of olefinic protons disappeared, and a new quintet signal (one proton) and two doublet of doublets (each two protons) at 3–4 ppm appeared. In the ^{13}C NMR spectra, two new signals in the aliphatic region (30–50 ppm) appeared, and olefinic signals were not observed. Finally according to the mass spectra of the isolated products, Michael addition of acetophenone to all the examined chalcone derivatives was confirmed (products **4a**–**4d**). The control reactions to find the progress of the reaction without enzyme (at room temperature and reflux in toluene) showed that lipase played a catalytic role in the carbon-carbon bond formation. Acetophenone formation from the initial substrate and further reaction by pyrrolidine as a base, which finally afforded a 1,4-addition compound, were observed in all reactions. The overall reactions had low yields; among them, substrates with electron-withdrawing groups had higher yields compared to electron-donating substituents.

In summary, the reaction concept was based on an initial Michael addition of pyrrolidine to benzylidene malonates but PPL catalyzed regiospecific aminolysis of benzylidene malonates, and the resulting product was the sole product without overaminolysis in spite of the excess amount of pyrrolidine. Also PPL is an efficient catalyst in the regiospecific amidation of benzylidene malonates. In spite of an excess amount of pyrrolidine, the *Z*-isomer was obtained as the sole product in moderate yields without overaminolysis. Also PPL catalyzed the Michael addition of acetophenone to chalcone derivatives.

EXPERIMENTAL

Diethylamine, pyrrolidine, piperidine, diethyl malonate, toluene, chloroform, hexane, and ethyl acetate (Merck) were used as purchased without further purification. Lipase from porcine pancreas lipase type II (PPL) (100–400 U/mg protein) was purchased from Sigma-Aldrich Co. and was used as supplied. Mass spectra were recorded on a Finnigan MAT TSQ-70 spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance 300 and 75 MHz, respectively. The spectra were taken in chloroform-d ($CDCl_3$) and the chemical shifts (δ) are given in ppm. The performance of enzymatic reactions was monitored by thin-layer chromatography (TLC).

General Procedure for Preparation of Chalcones. A mixture of acetophenone (1 mmol, 0.12 g), sodium hydroxide (5 mL, 3N), and ethanol (30 mL, 96%) was stirred at 0°C. 4-Chlorobenzaldehyde (1 mmol, 0.14 g) was added dropwise to the reaction mixture. After 2 h the product was isolated to obtain a yellow oil, which was re-crystallized from absolute ethanol to afford the pure chalcone. The other derivatives of benzaldehyde were used to prepare various chalcones.

General Procedure for Preparation of Benzylidene Malonates (1a–e). A mixture of diethyl malonate (6.5 mmol, 1 mL) and benzaldehyde (6.5 mmol, 0.69 mL) in 20 mL toluene was stirred at 110°C for 48 h. The reaction mixture was concentrated *in vacuo*, and the residual oil was purified by column chromatography to yield the corresponding pure benzylidene malonates.

General Procedure for Lipase-Catalyzed Aminolysis of Benzylidene Malonate Derivatives (2a–e). In a typical experiment, benzylidene malonate (1 mmol, 0.25 g) was added to 20 mL toluene. The reaction was initiated by adding 200 mg of lipase, and the mixture was further stirred at room temperature on an orbital shaker (120 rpm) for 1 h; then 0.2 mL pyrrolidine was added, and the reaction was monitored by TLC. The mixture was filtered, and the solvent of the filtrate was evaporated *in vacuo*. The resulting crude oily product was purified by thin-layer chromatography.

General Procedure for Lipase-Catalyzed Michael Addition of Acetophenone to Chalcone Derivatives (4a–d). To a mixture of 4-chlorochalcone (0.2 mmol, 0.048 g) in 20 mL toluene, 200 mg of PPL was added. The reaction mixture was stirred for 1 h. Then 0.2 mL pyrrolidine was added and the mixture stirred for a further 72 h after filtration from solid PPL. The product was purified by thin-layer chromatography.

Diethyl 2-Benzylidenemalonate (1a). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.74 (1H, s), 7.37–7.46 (5H, m), 4.33 (2H, q, J = 7.2), 4.30 (2H, q, J = 7.2), 1.33 (3H, t, J = 7.2), 1.28 (3H, t, J = 7.2).

Diethyl 2-(3-Chlorobenzylidene)malonate (1b). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.45 (1H, s), 7.30–7.38 (4H, m), 4.31 (2H, q, J = 7), 4.28 (2H, q, J = 7), 1.30 (3H, t, J = 7), 1.27 (3H, t, J = 7).

Diethyl 2-(3-Nitrobenzylidene)malonate (1c). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.31 (1H, s), 7.55–8.24 (4H, m), 4.35 (2H, q, J = 7.2), 4.31, (2H, q, J = 7.2), 1.32 (3H, t, J = 7.2), 1.30 (3H, t, J = 7.2).

Diethyl 2-(3-(Dimethylamino)benzylidene)malonate (1d). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.64 (1H, s), 7.38 (2H, d, J = 8.7), 6.65 (2H, d, J = 8.7), 4.38 (2H, q, J = 6.9), 4.28 (2H, q, J = 6.9), 3.04 (6H, s), 1.35 (3H, t, J = 6.9), 1.33 (3H, t, J = 6.9).

Diethyl 2-(3-Methoxybenzylidene)malonate (1e). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.08 (1H, s), 6.88–7.39 (4H, m), 4.30 (2H, q, J = 7.2), 4.27 (2H, q, J = 7.2), 3.85 (3H, s), 1.33 (3H, t, J = 7.2), 1.23 (3H, t, J = 7.2).

(*Z*)-Ethyl 3-Phenyl-2-(pyrrolidine-1-carbonyl)acrylate (2a). Yield 38%. IR (KBr, ν, cm^{-1}): 2968, 2872, 1713, 1634, 1444. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.68 (1H, s), 7.36–7.55 (5H, m), 4.32 (2H, q, J = 6.9), 3.63 (2H, m), 3.15 (2H, br.s), 1.78–1.90 (4H, m), 1.34 (3H, t, J = 7.1). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 165.46, 164.72, 140.20, 133.11, 130.46, 129.74, 128.90, 127.04, 61.48, 46.99, 45.51, 25.67, 24.41, 14.29.

(*Z*)-Ethyl 3-(3-Chlorophenyl)-2-(pyrrolidine-1-carbonyl)acrylate (2b). Yield 35%. IR (KBr, ν, cm^{-1}): 2975, 2866, 1713, 1633, 1495, 1450. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.61 (1H, s), 7.33–7.49 (4H, m), 4.31 (2H, q, J = 7.1), 3.61 (2H, t, J = 6.5), 3.13 (2H, br.s), 1.78–1.90 (4H, m), 1.33 (3H, t, J = 7.2). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 165.19, 164.50, 138.73, 136.49, 131.62, 130.80, 129.32, 129.22, 61.64, 47.04, 45.57, 25.71, 24.41, 14.29.

(*Z*)-Ethyl 3-(3-Nitrophenyl)-2-(pyrrolidine-1-carbonyl)-acrylate (2c). Yield 30%. IR (KBr, ν, cm^{-1}): 2924, 2846, 1713, 1627, 1529, 1441. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.65–8.39 (5H, m), 4.35 (2H, q, J = 7.1), 3.69 (2H, t, J = 6.6), 3.22 (2H, t, J = 6.3), 1.85–1.97 (4H, m), 1.37 (3H, t, J = 7.2). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 198.29, 164.48, 164.00, 148.60, 137.09, 135.30, 134.78, 131.88, 130.06, 124.71, 123.82, 61.97, 47.11, 45.66, 25.79, 24.39, 14.27.

(*Z*)-Ethyl 3-(3-(Dimethylamino)phenyl)-2-(pyrrolidine-1-carbonyl)acrylate (2d). Yield 22%. IR (KBr, ν, cm^{-1}): 2917, 2846, 1700, 1629, 1591. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.58 (1H, s), 7.41 (2H, d, J = 8.7), 6.62 (2H, d, J = 8.7), 4.27 (2H, m), 3.64 (2H, m), 3.24 (2H, m), 3.02 (6H, s), 1.90 (4H, m), 1.31 (3H, t, J = 7.2).

(*Z*)-Ethyl 3-(3-Methoxyphenyl)-2-(pyrrolidine-1-carbonyl)acrylate (2e). Yield 25%. IR (KBr, ν, cm^{-1}): 2968, 2872, 1712, 1633, 1482, 1442. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.02 (1H, s), 6.83–7.52 (4H, m), 4.24 (2H, m), 3.80 (3H, s), 3.40 (2H, m), 3.05 (2H, br.s), 1.69–1.93 (4H, m), 1.24 (3H, t, J = 7.2). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 165.78, 164.88, 157.93, 134.92, 131.67, 128.18, 122.13, 120.65, 110.66, 61.23, 55.53, 46.99, 45.87, 25.98, 25.60, 14.27.

(*E*)-3-(4-Chlorophenyl)-1-phenylprop-2-en-1-one (3a). Yellow crystals, mp 114–116°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.04 (2H, d, J = 8), 7.78 (1H, d, J = 16), 7.50–7.64 (6H, m), 7.41 (2H, d, J = 8).

(*E*)-3-(4-Fluorophenyl)-1-phenylprop-2-en-1-one (3b).Yellow crystals, mp 88–90°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.03 (2H, d, J = 8), 7.80 (1H, d, J = 16), 7.46–7.68 (6H, m), 7.13 (2H, t, J = 8.4).

(*E*)-3-(4-Methoxyphenyl)-1-phenylprop-2-en-1-one (3c). Yellow crystals, mp 60–62°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.00 (2H, d, J = 6.6), 7.79 (1H, d, J = 16), 7.25–7.62 (6H, m), 6.93 (2H, d, J = 8.4), 3.85 (3H, s).

(*E*)-3-(Naphthalen-2-yl)-1-phenylprop-2-en-1-one (3d). Yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.69 (1H, d, J = 15.3), 8.26–7.45 (13H, m).

3-(4-Chlorophenyl)-1,5-diphenylpentane-1,5-dione (4a). Yield 35%. IR (KBr, ν, cm^{-1}): 2917, 2859, 1675, 1595. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.23–7.98 (14H, m), 4.07 (1H, quin, J = 6.9), 3.50 (2H, dd, J = 16.8, 6.8), 3.47 (2H, dd, J = 16.8, 6.8). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 198.24, 142.30, 136.70, 133.26, 132.32, 128.91, 128.74, 128.67, 128.11, 44.74, 36.44. MS-EI (*m/z*, %): 363 (3), 319 (1), 268 (1), 243 (50), 105 (100), 77 (79), 51 (24).

3-(4-Fluorophenyl)-1,5-diphenylpentane-1,5-dione (4b). Yield 33%. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.94–7.98 (14H, m), 4.08 (1H, quin, J = 7), 3.50 (2H, dd, J = 16.7, 6.8), 3.33 (2H, dd, J = 16.7, 6.8). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 198.41, 139.40, 136.76, 133.22, 129.02, 128.92, 128.65, 128.13, 115.55, 115.27, 44.97, 36.41.

3-(4-Methoxyphenyl)-1,5-diphenylpentane-1,5-dione (4c). Yield 27%. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.81–7.98 (14H, m), 4.04 (1H, quin, J = 7), 3.49 (2H, dd, J = 16.5, 6.8), 3.32 (2H, dd, J = 16.5, 6.8). MS-EI (*m/z*, %): $[M + H]^+$ 359 (10), 239 (78), 134 (21), 105 (100), 77 (88), 51 (24).

3-(Naphthalen-2-yl)-1,5-diphenylpentane-1,5-dione (4d). Yield 25%. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.39–8.21 (14H, m), 5.03 (1H, quin, J = 6.9), 3.65 (2H, dd, J = 17.0, 7.3), 3.55 (2H, dd, J = 17.0, 7.3). MS-EI (*m/z*, %): $[M + H]^+$ 379 (24), 259 (45), 241 (9), 153 (30), 105 (100), 77 (75).

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