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Trypsin-Catalyzed One-Pot Multicomponent Synthesis of 4-Thiazolidinones

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Abstract A facile enzymatic one-pot multicomponent synthesis of 4-thiazolidinones was developed. The trypsin from porcine pancreas displayed great catalytic activity to promote the reaction of aldehyde, amine and mercaptoacetic acid with high yields showing a broad substrate specificity in this reaction. This trypsin-catalyzed multicomponent conversion method provided a novel strategy to synthesize thiazolidinones and expanded the application of trypsin in organic synthesis.

Keywords Enzymatic catalysis · Heterocycles · Cyclization · Multicomponent · Thiazolidinones

1 Introduction

4-Thiazolidinones are an important group of heterocyclic compounds, having diverse biological uses as antibacterial [1], anticonvulsant [2], anti-inflammatory [3, 4], FSH receptor agonist [5], anticancer [6], antiviral [7], antifungal [8], and antihistaminic activities [9, 10]. In view of the pharmacological significance of 4-thiazolidinones, much effort has been made to construct it [11]. Several synthetic methods are reported to prepare 4-thiazolidinones and most commonly used methods are cyclocondensation of thioureas with α-halo acid derivatives [12] and cyclocondensation of azomethines (Schiff bases) with mercaptoacetic acid [13] or its derivatives. In most of the synthetic methods, the reaction requires prolonged heating, multistep procedures or other harsh conditions. Therefore, developing a facile and mild method is still in demand.

Recently, much progress of enzymatic organic reactions has flourished [14–17]. For our continuous interest in the enzymatic synthesis, our group has previously reported enzymes as catalysts in Knoevenagel condensation [18], Mannich reaction [19], and synthesis of spirooxindole derivatives reactions [20]. However, natural enzymes, which are capable of catalyzing multicomponent reactions, are very scarce [21]. Herein we report a novel efficient method to synthesize 4-thiazolidinones catalyzed by trypsin from porcine pancreas (PPT) under mild conditions, which can be considered as an example of enzyme promiscuity as expand the potential of hydrolytic enzymes in organic synthesis [22–24].

2 Experimental

All reagents were purchased without further purification, unless otherwise indicated. All solvents were distilled prior to use. Reactions were performed in oven dried glassware. ^{1}H NMR was recorded on a Bruker Avance 400 spectrometer at 400 MHz in $CDCl_3$ using TMS as internal

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standard. IR spectra were recorded on a Bruker Equinox-55 spectrophotometer in the 4,000–400 cm^{-1} region. Elemental analyses were performed on an EA-1110 instrument. A Hewlett-Packard model 6890 gas chromatograph with a capillary column (HP-5) and flame-ionization detector was used to analyze the yields of products using tridecane as an internal standard. Melting points were recorded on an X_4-Data microscopic melting point apparatus and were uncorrected. All the enzymes were purchased from Aldrich and used directly. The enzymatic units of all the enzymes are described below: PPT (2,500 units/mg), Lipase AY30 (30 units/mg), Lipase from porcine pancreas (≥200 units/mg), Diastase from *Aspergillus oryzae* (≥3.5 units/mg), α-Amylase from *Aspergillus oryzae* (∼30 units/mg), α-Amylase from hog pancreas (10 units/mg), Amano lipase M from *Mucor Javanicus* (10 units/mg).

General procedure for synthesis of 4-thiazolidinones: The mixture of 1.0 mmol amines, 1.0 mmol aldehyde, 1.0 mmol mercaptoacetic acid, 20 mg trypsin from porcine pancreas and 5 mL dichloromethane, was introduced into a test tube (10 mL), then the mixture was shaken at 160 rpm end-over-end rotation at 35 °C for 4 h. The reaction mixture was monitored by TLC to end (hexane/AcOEt = 4:1). The residue was purified on silica gel to afford the target compounds.

3 Result and Discussion

In order to find the most suitable enzyme for the envisaged one-pot synthesis of 4-thiazolinones reaction, we tested the reaction of 3-nitrobenzaldehyde **1a** (1 mmol), benzylamine **2a** (1 mmol), and mercaptoacetic acid **3a** (1 mmol) as model substrates. Various enzymes were employed in this reaction (Scheme 1 and Table 1). It was found that several enzymes displayed observable catalytic activities for this reaction. PPT showed an excellent catalytic activity, Lipase AY30, and Lipase from porcine pancreas also showed good catalytic activities (Entry 2, 3, Table 1). To determine the catalytic effects coming from enzyme, according to the literature [22], the blank control and inactivated enzyme experiments were performed (Entry 9, 10, Table 1) and only trace amount of product was detected. The results demonstrate that PPT played an important catalytic role in this reaction which maybe attribute to its unique steric and electronic effects from the catalytic site.

Table 1 Optimization of catalyst

Entry	Catalyst	Yield (%)[a]
1	Trypsin from porcine pancreas (PPT)	96
2	Lipase AY30	85
3	Lipase from porcine pancreas	82
4	Diastase from *Aspergillus oryzae*	75
5	α-Amylase from *Aspergillus oryzae*	73
6	α-Amylase from hog pancreas	64
7	Amano lipase M from *Mucor javanicus*	63
8	Bovine serum albumin (BSA)	60
9	Trypsin from porcine pancreas (inactivated)[b]	Trace
10	Blank	Trace

Reaction conditions: benzylamine (1 mmol), 3-nitrobenzaldehyde (1 mmol), and mercaptoacetic acid (1 mmol), trypsin from porcine pancreas (20 mg), dichloromethane (5 mL), shaken at 160 rpm at 35 °C for 4 h

[a] GC yields are based on tridecane as an internal standard

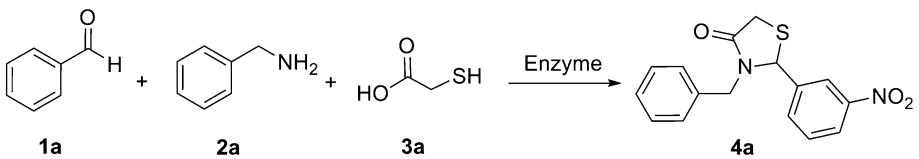
[b] Trypsin from porcine pancreas denatured with urea at 100 °C for 10 h [25]

The reaction medium has been recognized to be one of the most important factors influencing the enzymatic reaction. Several solvents were used to explore the best solvent for this reaction. The results are shown in Table 2, which demonstrated that this reaction went smoothly in the presence of CH_2Cl_2 with the highest yield (Entry 1, Table 2) compared to other solvents. From the view of solvent polarity, non-polar solvents are better than polar solvents which maybe attributes that polar solvents have a tendency to strip off constitutive water molecules from the inner structure of the enzyme, resulting in its unfolding and reducing catalytic activity.

In order to improve the activity of enzyme, other influencing factors such as temperature, concentration of catalyst, and reaction time have also been investigated (Table 3). It was found that the temperatures ranged from 25 to 40 °C, the yield of the products increased when the enzyme amount is 20 mg. While the enzyme amount exceeded 30 mg, the yields decrease (Entry 3–7, Table 3) which maybe attributes to the reunion of enzyme when its concentration is high in solution. Synthetically, the optimum reaction condition is 20 mg PPT in 5 mL solvent at 35 °C/4 h.

In order to expand the substrates, different amines and aldehydes were employed (Scheme 2 and Table 4). The electronic and steric effects are investigated by employing

Scheme 1 The model reaction of one-pot multicomponent enzymatic synthesis of 4-thiazolidinones 148 × 25 mm



electron-donating or -withdrawing groups and different bulking groups. The results revealed that all the aldehydes and amines reacted well and give 4-thiazolidinones derivatives with good yields. It seemed that the enzymes have a wide tolerance range towards aldehydes and amines in this reaction.

Table 2 Trypsin-catalyzed the reaction of 3-nitrobenzaldehyde, benzylamine and mercaptoacetic acid in different solvents

Entry	Solvent	T/ °C	Yield (%)[a]
1	CH_2Cl_2	35	96
2	*n*-Hexane	35	55
3	1,4-Dioxane	35	50
4	Ethanol	35	43
5	CH_3CN	35	42
6	THF	35	37
7	Methanol	35	23
8	Acetone	35	11
9	Water	35	Trace

Reaction conditions: benzylamine (1 mmol), 3-nitrobenzaldehyde (1 mmol), and mercaptoacetic acid (1 mmol), trypsin from porcine pancreas (20 mg), solvent (5 mL), shaken at 160 rpm at 35 °C

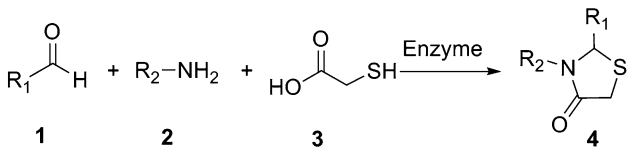
[a] GC yields are based on tridecane as an internal standard

Table 3 Optimization of the reaction conditions

Entry	PPT amount (mg)	Temp (°C)	Time (h)	Yield (%)[a]
1	20	25	4	76
2	20	30	4	80
3	20	40	4	97
4	10	35	4	77
5	30	35	4	90
6	40	35	4	88
7	50	35	4	85
8	20	35	3	76
9	20	35	4	96
10	20	35	5	96

Reaction conditions: benzylamine (1 mmol), 3-nitrobenzaldehyde (1 mmol), and mercaptoacetic acid (1 mmol), trypsin from porcine pancreas (20 mg), dichloromethane (5 mL), shaken at 160 rpm

[a] GC yields are based on tridecane as an internal standard



Scheme 2 Trypsin-catalyzed one-pot multicomponent synthesis of 4-thiazolidinones 99 × 23 mm

Table 4 Synthesis of 4-thiazolidinones in trypsin-catalyzed reaction between different amines, aldehydes and mercaptoacetic acid

Entry	Products	R_1	R_2	Yield (%)
1	**4a**	3-Nitrophenyl	Benzyl	96[a] (87[b])
2	**4b**	3-Nitrophenyl	2-Propynyl	81 (71)
3	**4c**	4-Chlorophenyl	Benzyl	70 (60)
4	**4d**	4-Chlorophenyl	2-Propynyl	74 (61)
5	**4e**	3-Methylphenyl	Benzyl	83 (70)
6	**4f**	3-Methylphenyl	2-Propynyl	88 (72)
7	**4g**	4-Methoxyphenyl	Benzyl	55 (42)
8	**4h**	4-Methoxyphenyl	2-Propynyl	72 (65)
9	**4i**	4-Hydroxyphenyl	Benzyl	54 (48)
10	**4j**	4-Hydroxyphenyl	2-Propynyl	77 (65)
11	**4k**	2-Methoxyphenyl	Benzyl	90 (81)
12	**4l**	2-Methoxyphenyl	2-Propynyl	70 (64)
13	**4m**	2-Naphthyl	Benzyl	78 (67)
14	**4n**	2-Naphthyl	2-Propynyl	76 (60)
15	**4o**	3-Benzonitrile	Benzyl	69 (55)
16	**4p**	3-Benzonitrile	2-Propynyl	74 (67)
17	**4q**	3-Pyridyl	Benzyl	98 (89)
18	**4r**	3-Pyridyl	2-Propynyl	88 (79)
19	**4s**	2-Nitrophenyl	Benzyl	84 (76)
20	**4t**	2-Nitrophenyl	2-Propynyl	79 (68)

Reaction conditions: amines (1 mmol), aldehyde (1 mmol), and mercaptoacetic acid (1 mmol), trypsin from porcine pancreas (20 mg), dichloromethane (5 mL), shaken at 160 rpm at 35 °C

[a] GC yields are based on tridecane as an internal standard

[b] Isolated yields

4 Conclusions

In conclusion, an efficient enzymatic one-pot multicomponent synthesis of 4-thiazolidinones was developed and the reaction conditions were performed. Trypsin from porcine pancreas (PPT) displayed great catalytic activity in this reaction and showed a wide tolerance range towards substrates aldehydes and amines. This trypsin-catalyzed multicomponent conversion method provided a novel strategy and useful tool for the synthesis of thiazolidinones and expand the toolbox for synthetic chemists.

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