

D-Aminoacylase-initiated cascade Aldol condensation/Robinson annulation for synthesis of substituted cyclohex-2-enones from simple aldehydes and acetone

Ziwei Xiang · Yiru Liang · Xiang Chen ·
Qi Wu · Xianfu Lin

Received: 16 January 2014 / Accepted: 7 April 2014 / Published online: 27 April 2014
© Springer-Verlag Wien 2014

Abstract As an important building block, developing efficient and green synthesis strategy of cyclohex-2-enones is of great importance. In this present work, a general approach to the mild synthesis of substituted cyclohex-2-enones derivatives starting from simple aldehydes and acetone have been achieved via D-aminoacylase-initiated Aldol condensation/Robinson annulation cascade reaction using imidazole as an additive in organic media. The influences of reaction conditions including solvents, enzyme concentration, additives type, molar ratio of enzyme to additive, and substrate scopes were systematically investigated. Furthermore, some experiments were designed to explore the catalytic roles of D-aminoacylase and imidazole in the multistep cascade process, and one possible mechanism was proposed.

Keywords Cascade reaction · D-Aminoacylase · Imidazole · Substituted cyclohex-2-enones

Introduction

Compounds containing cyclohex-2-enone (Fig. 1) have been considered as an important class of organic compounds, not only because of its successful application as a building block in the natural synthesis, but numerous

compounds with this scaffold have also exhibited significant biological activities and are widely utilized as pheromone (Mori et al. 1978; Plummer et al. 1976; Ross et al. 1996; Vité et al. 1972), food additive (Authority 2011) or antitumor (Nakayachi et al. 2004). Many works have focused on the development of novel and efficient synthetic routes for the preparation of cyclohex-2-enones derivatives and continuing studies are in progress. So far, a variety of methods have been established, including Hagemann's or Knoevenagel's approach (Horning et al. 1944; Horning and Field 1946; Pollini et al. 2010; Smith and Eftax 1956), reductive cyclization of 2,6-dimethyl-3,5-dicarboxyethyl-4-aryl-1,4-dihydropyridines (Martínez et al. 1998), intramolecular aldolization of 4-substituted 2,6-heptandiones (Zhou et al. 2008). However, they usually suffered from harsh reaction conditions, longer synthesis step or low yields. Therefore, developing an efficient and green approach for synthesis of cyclohex-2-enone derivatives using commercially available and simple materials is of great use and much interest.

The usefulness of various biocatalysts (such as enzymes or whole cells) for organic synthesis has attracted more and more attention from chemists and biochemists due to mild reaction conditions, wide sources, broad range of substrates and potential use of inexpensive regenerable resources (Aleu et al. 2006; Feng et al. 2009; Kazlauskas 2005; Pollard and Woodley 2007; Schmid et al. 2001; Wu et al. 2010). Particularly, enzymatic promiscuity, i.e., the ability of a single active site of the enzyme to catalyze alternative reactions that differ from their natural physiological reaction, has been a subject of increasing research interest in the recent few years (Baas et al. 2013; Busto et al. 2010; Khersonsky and Tawfik 2010). Some promiscuous hydrolases-catalyzed carbon–carbon (Cai et al. 2011; Svedendahl et al. 2005; Xu et al. 2007) and carbon–heteroatom (Carlqvist et al. 2005; Kitazume et al. 1986; Lou et al. 2008;

Electronic supplementary material The online version of this article (doi:10.1007/s00726-014-1747-6) contains supplementary material, which is available to authorized users.

Z. Xiang · Y. Liang · X. Chen · Q. Wu (✉) · X. Lin (✉)
Department of Chemistry, Zhejiang University,
310027 Hangzhou, People's Republic of China
e-mail: wuqi1000@163.com

X. Lin
e-mail: llc123@zju.edu.cn

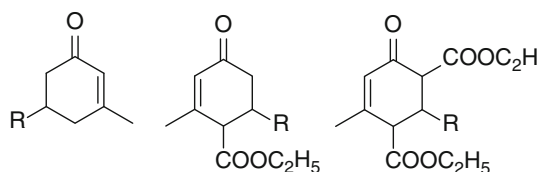
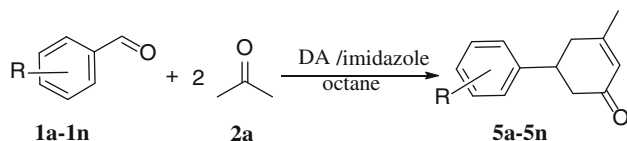


Fig. 1 Compounds containing cyclohex-2-enone



Scheme 1 Enzymatic cascade reaction of aldehydes and acetones produces 5-aryl-3-methylcyclohex-2-enones

Torre et al. 2004; Wu et al. 2005, 2006) bond-forming reactions, such as Aldol reaction (He et al. 2012; Guan et al. 2012) and Michael reaction (Wang et al. 2009), have been done during the past decade. Although many promiscuous enzyme-catalyzed transformations have been reported, only a handful of enzymatic cascade reactions have been described (Liu et al. 2013; Wu et al. 2011; Zhou et al. 2013). For example, Renard and co-workers reported that Lipozyme[®] could catalyze the acylation/cyclization reactions between fatty acid and *o*-phenylenediamine to give 2-alkyl-benzimidazoles in mild yields, and a further study was also reported by the group of Yu (Renard and Lerner 2007; Wang et al. 2010). Zhang et al. reported a multistep cascade reaction, which was catalyzed by PPL in the presence of water, producing spirooxindole derivatives in excellent yields (Chai et al. 2011). Our group have also developed a direct approach to 1,4-dihydropyridines by lipase-catalyzed Hantzsch-type reaction starting from aldehydes, 1,3-dicarbonyl compounds and acetamide in one pot (Wang et al. 2011a).

As a part of our continuous efforts toward the development of biocatalytic promiscuity, we are interested in the study of enzyme-catalyzed synthesis of cyclic compounds through cascade reactions, and fortunately found that D-aminoacylase could catalyze Aldol–Robinson cascade reaction between simple aldehydes and acetones to produce 5-aryl-3-methylcyclohex-2-enones in the presence of imidazole. Although Zhao et al. and our group have found that 5-aryl-3-methylcyclohex-2-enones were directly synthesized from aldehydes and acetone using pyrrolidine/propionic acid or lysine/imidazole as catalyst (Wang et al. 2011b; Xiang et al. 2013), no enzymatic methods for preparation of 5-aryl-3-methylcyclohex-2-enones were reported. In the present work, we reported a facile and useful enzymatic strategy for the synthesis of 5-aryl-3-

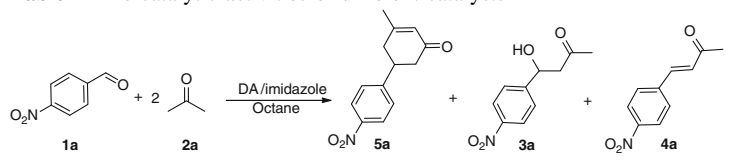
methylcyclohex-2-enones in organic media in the presence of imidazole (Scheme 1).

Results and discussion

In initial research, we chose *p*-nitrobenzaldehyde **1a** and acetone **2a** as the model substrates, and investigated the reaction between them under the catalysis of a series of commercially available enzymes. The results showed that a zinc-binding metallo-acylase, D-aminoacylase from *Escherichia coli*, which naturally catalyzes the hydrolysis of *N*-acyl-D-amino acids (Lin et al. 2007, 2009), displayed the best activity (Table S1, entry 7). Although the main products of this reaction catalyzed by D-aminoacylase were aldol product (**3a**, 34 %) and Knoevenagel condensation product (**4a**, 56 %), it was found that 8 % targeted product **5a** could be obtained after 48 h (Table 1, entry 2 and Table S1, entry 7). Some other hydrolases, such as CAL-B, Lipozyme[®], PPL, HPL, PGA, and CCL, were also investigated and all of them exhibited no catalytic activity for synthesis of **5a** (Table S1, entries 1–6). Subsequently, based on our previous research that the introduction of organic molecules as additive had a great influence on the results of biocatalytic reactions (Chen et al. 2011), we then designed some experiments to explore the effect of different additives and found that the yield of **5a** improved from 8 to 27 % when 0.22-mmol imidazole was added to the reaction system (Table S2, entries 1–9). In addition, it could be also found that main product of this reaction could be regulated by imidazole; for example, the main product of this reaction without any additive were aldol product **3a** in the presence of D-aminoacylase, while the introduction of 10-mg imidazole (0.22 mmol) to the reaction system leading to the Knoevenagel condensation compound **4a** as main product after 12 h (Table 1, entry 1 versus entry 3), and the yield of 5-aryl-3-methylcyclohex-2-enone (**5a**) was improved from 8 to 27 % after 48 h (Table 1, entry 2 versus entry 4).

Furthermore, some controlled experiments were performed to demonstrate the specific catalytic effect of D-aminoacylase. When the reaction was incubated with BSA or denatured D-aminoacylase (pretreated with urea at 100 °C for 8 h or pretreated with EDTA at 37 °C for 12 h), or imidazole alone, no product was detected (Table 1, entries 5–8). Without doubt, the reaction between aldehyde and acetone could not occur without any catalysts (Table 1, entries 9). These experimental results indicated that D-aminoacylase-imidazole had the catalytic ability for the cascade reaction.

Considering the reaction may be affected by the loading amount of imidazole, corresponding experiments were carried out. As shown in Fig. 2, it could be observed that

Table 1 The catalytic activities of different catalysts


Entry	Enzyme	Imidazole (mmol)	Time (h)	Yield (%) ^c 3a	Yield (%) ^c 4a	Yield (%) ^c 5a
1	D-Aminoacylase	—	12	81	13	N.D. ^d
2	D-Aminoacylase	—	48	56	34	8
3	D-Aminoacylase	0.22	12	9	73	12
4	D-Aminoacylase	0.22	48	3	70	27
5	—	0.22	48	7	5	N.D
6	D-Aminoacylase denatured with urea ^a	0.22	48	10	7	N.D
7	D-Aminoacylase denatured with EDTA ^b	0.22	48	12	6	N.D
8	BSA	—	48	9	7	N.D
9	No catalyst	—	48	N.D	N.D	N.D

Reaction conditions: enzyme (25 mg), *p*-nitrobenzaldehyde (0.07 mmol), acetone (0.15 mL), octane (1 mL), 50 °C

^a Pretreated with urea at 100 °C for 8 h

^b Pretreated with EDTA at 37 °C for 12 h

^c Yields were determined by HPLC

^d Not detected

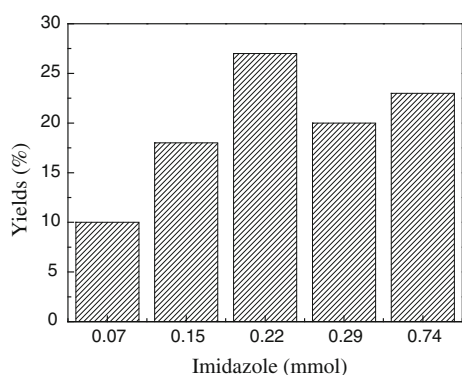


Fig. 2 The influence of imidazole on the cascade reaction. Reaction conditions: D-aminoacylase (25 mg), *p*-nitrobenzaldehyde (0.07 mmol), acetone (0.15 mL), solvent (1 mL), 50 °C, 48 h; yields were determined by HPLC

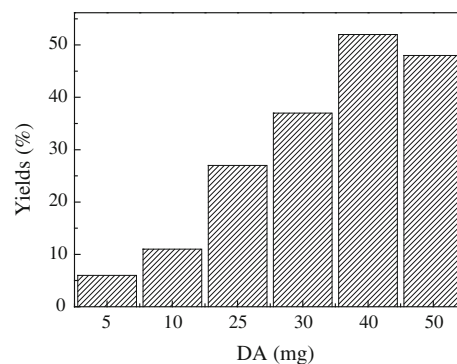


Fig. 3 The influence of D-aminoacylase on the cascade reaction. Reaction conditions: imidazole (0.22 mmol), *p*-nitrobenzaldehyde (0.07 mmol), acetone (0.15 mL), solvent (1 mL), 50 °C, 48 h; yields were determined by HPLC

the yields reached the maximum when the imidazole loading amount was 0.22 mmol, and had no further improvement while increasing the dosage of imidazole. The influence of enzyme concentration on the reaction efficiency was also investigated. According to the data shown in Fig. 3, with the concentration of D-aminoacylase changed from 5 to 50 mg/mL, the yield increased from 6 to 52 % and then decreased slightly. Therefore, the optimal enzyme and imidazole concentration were 40 mg/mL and 0.22 mmol/mL, respectively.

The reaction medium is an important factor in the enzymatic reaction, due to the fact that enzyme may have

slightly different conformation in different media and thus show distinct catalytic activities (Dhake et al. 2010; Li et al. 2009). To improve the activity of D-aminoacylase, some conventional organic solvents with different log *p* values were screened and the results are shown in Table 2. It could be observed that octane was the most efficient medium for this reaction, and the product was obtained in yield of 52 % (Table 2, entry 4). Surprisingly, we found that other low polar solvents, such as hexane and toluene, showed no promotion for the reaction and gave the targeted product **5a** with yields <5 % (Table 2, entries 1–3). While the reactions could not occur in polar solvents,

Table 2 The influence of solvent on the cascade reaction of **1a** and acetone

Entry	Solvent	Log <i>p</i>	Yield (%) ^a
1	Hexane	3.9	4
2	1,4-Dioxane	−0.5	1
3	Toluene	2.6	<1
4	Octane	4.9	52
5	<i>N,N</i> -Dimethylformamide	−1.0	N.D ^b
6	CHCl ₃	2.0	N.D
7	DMSO	−1.3	<1
8	NMP ^c	–	N.D
9	EtOH	–	N.D
10	H ₂ O	–	<1

Reaction conditions: D-aminoacylase (40 mg), *p*-nitrobenzaldehyde (0.07 mmol), acetone (0.15 mL), imidazole (0.22 mmol), solvent (1 mL), 50 °C, 48 h

^a Yields were determined by HPLC

^b Not detected

^c *N*-methyl-2-pyrrolidone

no matter whether protic or aprotic solvent, this may be due to that polar solvents lower the catalyst activity of D-aminoacylase (Table 2, entries 5–10). Thus, octane was chosen as the optimized medium for further investigation.

Based on the above optimal reaction conditions, some other aldehydes bearing different substituents (**1a–n**) were selected to expand the generality and scope of this new D-aminoacylase/imidazole-catalyzed cascade reaction. These results were summarized in Table 3. Generally speaking, substitution with strong electron-withdrawing groups, such as $-\text{CF}_3$, $-\text{CN}$, $-\text{NO}_2$, could enhance the reactivity of the substrate and lead to good yields (Table 3, entries 1, 2 and 8–10), compared with electron-donating substituents (Table 3, entries 12, 13). The reason for this result might be the increase in the electron density of carbonyl carbon atom as a result of the strong electron-donating effect of electron-donating groups, thus reducing the reactivity of the carbonyl group. Meanwhile, it was found that halogen atom ($-\text{F}$, $-\text{Cl}$, $-\text{Br}$) in aromatic ring might have an influence on the catalyst activity of D-aminoacylase and could cause the decrease of yields (Table 3, entries 3–7). Interestingly, the cascade reaction between benzaldehyde and acetone could give final product **5k** with a good yield of 70 %, possibly due to smaller steric hindrance in the active site of D-aminoacylase (Table 3, entry 11). On other hand, we have also investigated the reaction between *p*-nitrobenzaldehyde and unsymmetrical ketones (such as 2-butanone and 3-pentanone), it was found that only aldol adducts were obtained and no targeted products could be detected. This may be because D-aminoacylase has the limited spatial structure, and possibly the steric hindrance

arisen from aldol adducts and unsymmetrical ketones could not fit well with the binding pocket of D-aminoacylase, which resulted in the failure of the Robinson annulations reaction between aldol adducts and unsymmetrical ketones. Moreover, this reaction between heterocyclic aldehyde and acetone also gave the desired product with moderate yield (Table 3, entry 14).

Next, considering the multistep feature and complexity of this D-aminoacylase/imidazole-catalyzed cascade reaction, one important issue should be discussed, namely what catalytic roles the D-aminoacylase and imidazole played, respectively, in these multisteps. As shown in Table 1, the model reaction between *p*-nitrobenzaldehyde and acetone can provide aldol product (**3a**), Knoevenagel condensation product (**4a**), and the targeted product (**5a**). According to our hypothesis, the cascade reaction possibly went through several simple reaction processes such as aldol reaction, dehydration (Knoevenagel condensation) and Robinson annulations, and the detailed mechanism was discussed in the next section. In order to further comprehend the role of both D-aminoacylase and imidazole in each step of the cascade reaction, some additional experiments were needed.

In the model reaction shown in Table 4, when D-aminoacylase alone was used as the catalyst, the aldol product **3a** was obtained in a yield of 80 %, while the Knoevenagel condensation product (**4a**) was <10 % (Table 4, entry 3). Compared with the reaction catalyzed by apoenzyme, which was obtained from D-aminoacylase, the results clearly showed that D-aminoacylase exhibited a good catalytic promiscuity for aldol reaction (Table 4, entry 3 versus entry 2), which was in accord with our previous results (Chen et al. 2011). Moreover, the addition of imidazole greatly increased the yield of Knoevenagel condensation product (**4a**) (from 6 % in entry 3 to 68 % in entry 5, Table 4), which implied that imidazole possibly accelerated the dehydration process of aldol product (**3a**) to give Knoevenagel condensation product (**4a**). By the way, imidazole had no catalytic activity for the aldol reaction (Table 4, entry 1).

The role of imidazole in the dehydration process was further verified by the following experiment, the dehydration of the intermediate product **3a** (Table 5). Imidazole exhibited strong ability of promoting the dehydration of **3a**, especially when adding the acetone to increase the dissolution of **3a**, the highest yield of 78 % for **4a** could be obtained (Table 5, entries 1, 2). D-Aminoacylase also showed certain activity for the dehydration of **3a** to give compound **4a** (Table 5, entries 3, 4), and the addition of acetone led to small amount of final product (**5a**). It is noteworthy that no final product was detected when acetone was added to the imidazole-catalyzed dehydration of **3a** (Table 5, entry 2), which primarily implied that

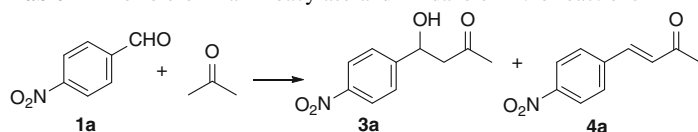
Table 3 D-Aminoacylase-catalyzed cascade reaction of aldehyde and acetone

Entry	Substrate	Products	Yields (%) ^[a]
1			52
2			74
3			30
4			37
5			45
6			42
7			38
8			67
9			73
10			74
11			70
12			21
13			N.D. ^[c]
14			32

Reaction conditions:
D-aminoacylase (40 mg),
aldehyde (0.07 mmol), acetone
(0.15 mL), imidazole (0.22
mmol), octane (1 mL), 50 °C,
48 h

^a Yields were determined by
HPLC

^b Not detected

Table 4 The role of D-aminoacylase and imidazole in the reactions

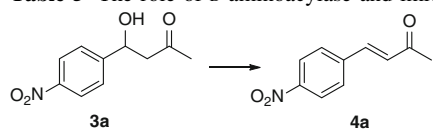
Entry	D-Aminoacylase (mg)	Imidazole (mmol)	Yields (%) 3a	Yields (%) ^a 4a
1	–	0.22	N.D. ^c	N.D
2	40 ^b	–	N.D	N.D
3	40	–	80	6
4	–	–	N.D	N.D
5	40	0.22	N.D	68

Reaction conditions: **1a** (0.07 mmol), octane (1 mL), acetone (0.15 mL), 50 °C, 12 h

^a Yields were determined by HPLC

^b Metal-free apoenzyme

^c Not detected

Table 5 The role of D-aminoacylase and imidazole in the process of dehydration

Entry	D-Aminoacylase (mg)	Imidazole (mmol)	Acetone (mL)	4a Yields (%) ^a
1	–	0.22	–	12
2	–	0.22	0.15	78
3	40	–	–	16
4	40	–	0.15	30 (5a : 8)
5	40 ^b	–	0.15	N.D. ^c
6	40	0.22	–	37
7	40	0.22	0.15	38 (5a : 61)
8	40 ^b	0.22	–	9
9	–	–	–	N.D

Reaction conditions: **3a** (0.05 mmol), octane (1 mL), 50 °C, 12 h

^a Yields were determined by HPLC

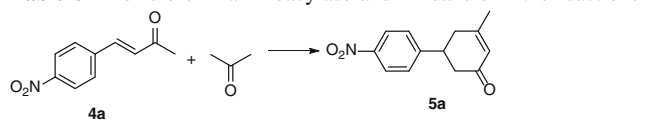
^b Metal-free apoenzyme

^c Not detected

imidazole had no activity for the subsequent process of Robinson annulation. Without doubt, the combination of imidazole and D-aminoacylase catalyzed the dehydration of **3a** more efficiently than independent one (Table 5, entries 6 and 7). Especially, the addition of acetone promoted the synthesis of final product **5a** greatly (61 % yield). Control reactions catalyzed by metal-free apoenzyme or without catalyst further confirmed the catalytic role of imidazole and D-aminoacylase (Table 5, entries 5, 8 and 9).

Furthermore, we investigated the Robinson annulation of compound **4a** and acetone, namely a cascade Michael addition/intramolecular aldol condensation process. As shown in Table 6, it was found that no product was detected when imidazole or apoenzyme was used as the

catalyst, respectively, possibly due to the fact that imidazole or apoenzyme had no catalytic activity for some key step in the Robinson annulations such as the step of Michael addition (Table 6, entries 1, 2, 3, and 5) (Xu et al. 2007). While D-aminoacylase alone showed certain activity for this Robinson annulation, and 10 % of **5a** was detected (Table 6, entry 1). Interestingly, the addition of imidazole greatly improves the efficiency of the D-aminoacylase-catalyzed Robinson annulations, and 72 % yield of **5a** was obtained (Table 6, entry 4). One possible reason for the enhancement by imidazole addition was that imidazole showed high activity for the dehydration process in the Robinson annulations similar as in the Knoevenagel condensation.

Table 6 The role of D-aminoacylase and imidazole in the reactions


Entry	D-Aminoacylase (mg)	Imidazole (mmol)	Yields ^a
1	40	—	10
2	40 ^c	—	<1
3	—	0.22	N.D. ^b
4	40	0.22	72
5	40 ^c	0.22	<1

Reaction conditions: **4a** (0.05 mmol), acetone (0.15 mL), octane (1 mL), 50 °C, 24 h

^a Yields were determined by HPLC

^b Not detected

^c Metal-free apoenzyme

Based on the above experimental results and some previous works concerning the catalytic mechanism of promiscuous zinc-dependent acylase (Liu et al. 2013; Xu et al. 2007), one possible mechanism of the synthesis of 3-methyl-5-aryl-2-cyclohexen-1-ones catalyzed by D-aminoacylase in the presence of imidazole was proposed (Scheme 2). The zinc ion would coordinate with the carbonyl group of acetone, leading to render the acetone more nucleophilic. Then Asp366 deprived one H of acetone, and the nucleophile would add simultaneously to the carbonyl group of aldehyde. Then one water molecule was lost to form the Knoevenagel condensation product **4a**. Next, the Michael reaction between **4a** and acetone occurred to form intermediate **6**, which was quickly converted to intermediate **7** through intramolecular aldol reaction because of the combined action of Zn²⁺ and Asp366. At last, intermediate **7** lost one molecular water to get the final product **5a**. It

should be remarked that the dehydration process in the cascade reaction was the catalyzing results of D-aminoacylase and imidazole.

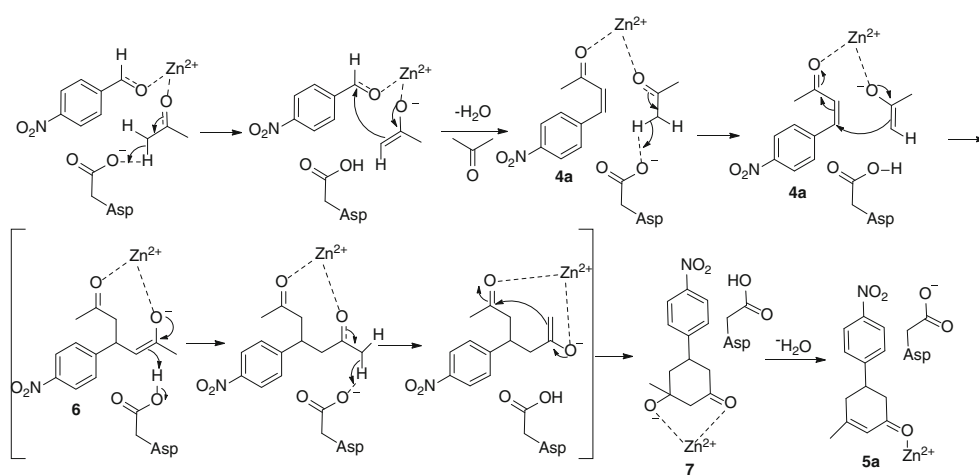
In conclusion, a novel one-pot enzymatic method to construct 3-methyl-5-aryl-2-cyclohexen-1-ones via cascade aldol condensation/Robinson annulation of aromatic aldehyde and acetone was first reported, using D-aminoacylase as catalyst and imidazole as additive. This protocol provides an easy-operating and efficient synthetic route of 3-methyl-5-aryl-2-cyclohexen-1-one derivatives from simple aldehydes and acetones. It works with a wide range of substrates with moderate to good yields. A series of experiments were designed to explore the catalytic roles of D-aminoacylase and imidazole in this cascade process. More importantly, this simple enzymatic cascade reaction provided a novel case of catalytic promiscuity which reinforces the utilization of enzyme in organic synthesis and the discovery of pharmaceutical structures.

Experimental section

Materials and general methods

D-Aminoacylase from *E. coli* (10,000 U/mg, 1 U is defined as enzyme quantity, which produces 1 mmol of D-amino acid per 30 min) and Acylase Amano from *Aspergillus oryzae* ($\geq 30,000$ U/g, 1 U is defined as enzyme quantity, which produces 1 mmol of L-Amino acid per 30 min) were purchased from Amano Enzyme Inc (Japan). Immobilized penicillin G acylase from *E. coli* (EC 3.5.1.11, immobilized on acrylic beads) was purchased from Hunan Flag Biotech Co. All chemicals were obtained from commercial suppliers and used without further purification. Unless otherwise noted, all commercially available compounds were used without further purification.

Scheme 2 Proposed mechanism of the synthesis of 3-methyl-5-aryl-2-cyclohexen-1-ones



The ^1H and ^{13}C NMR spectra were recorded with TMS as internal standard using a Bruker AMX-400 MHz spectrometer. Chemical shifts were expressed in parts per million and coupling constants (J) in Hertz. IR spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. All the known products were characterized by comparing the ^1H NMR data with those reported in the literature. The structures of new compounds were confirmed by IR, ^1H NMR, ^{13}C NMR, and HR-MS. Analytical HPLC was performed using an Agilent 1100series with a reversed-phase Shim-PackVP-ODS column (150 \times 4.6 mm) and a UV detector (250 nm).

General procedure for the enzymatic cascade for synthesis of 5-aryl-3-methylcyclohex-2-enones

A suspension of **1a–n** (0.7 mmol), acetone (1.5 mL), 2.1-mmol imidazole and 400*mg D-aminoacylase in 10-mL octane was incubated at 50 °C and shaken for 48 h. After the indicated time, the enzyme was filtered off to terminate the reaction and solvent was evaporated under vacuum to dryness. The crude residue was purified by flash chromatography on silica gel using petroleum/ethyl acetate mixtures. Product-containing fractions were combined, concentrated, and dried to give **5a–n**.

Acknowledgments The authors gratefully acknowledge the financial support from National Natural Science Foundation of China (No. 21072172, 21272208).

Conflict of interest We declare that we have no competing financial interests.

References

- Aleu J, Bustillo AJ, Hernandez-Galan R, Collado IG (2006) Biocatalysis applied to the synthesis of agrochemicals. *Curr Org Chem* 10(16):2037–2054
- Authority EFS (2011) Scientific opinion on flavouring group evaluation 212: α,β -unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE. 19. EFSA J 9(3):1923
- Baas BJ, Zandvoort E, Geertsema EM, Poelarends GJ (2013) Recent advances in the study of enzyme promiscuity in the tautomerase superfamily. *Chem Biol Chem* 14(8):917–926
- Busto E, Gotor-Fernandez V, Gotor V (2010) Hydrolases: catalytically promiscuous enzymes for non-conventional reactions in organic synthesis. *Chem Soc Rev* 39(11):4504–4523
- Cai JF, Guan Z, He YH (2011) The lipase-catalyzed asymmetric C–C Michael addition. *J Mol Catal B Enzym* 68(3–4):240–244
- Carlqvist P, Svedendahl M, Branneby C, Hult K, Brinck T, Berglund P (2005) Exploring the active-site of a rationally redesigned lipase for catalysis of Michael-type additions. *Chem Biol Chem* 6(2):331–336
- Chai SJ, Lai YF, Xu JC, Zheng H, Zhu Q, Zhang P (2011) One-pot synthesis of spirooxindole derivatives catalyzed by lipase in the presence of water. *Adv Synth Catal* 353(2–3):371–375
- Chen X, Liu BK, Kang H, Lin XF (2011) A tandem Aldol condensation/dehydration co-catalyzed by acylase and *N*-heterocyclic compounds in organic media. *J Mol Catal B Enzym* 68(1):71–76
- Dhake KP, Tambade PJ, Singhal RS, Bhanage BM (2010) Promiscuous candida antarctica lipase B-catalyzed synthesis of β -amino esters via aza-Michael addition of amines to acrylates. *Tetrahedron Lett* 51(33):4455–4458
- Feng XW, Li C, Wang N, Li K, Zhang WW, Wang Z, Yu XQ (2009) Lipase-catalysed decarboxylative aldol reaction and decarboxylative Knoevenagel reaction. *Green Chem* 11(12):1933–1936
- Guan Z, Fu JP, He YH (2012) Biocatalytic promiscuity: lipase-catalyzed asymmetric aldol reaction of heterocyclic ketones with aldehydes. *Tetrahedron Lett* 53(37):4959–4961
- He YH, Li HH, Chen YL, Xue Y, Yuan Y, Guan Z (2012) Chymopapain-catalyzed direct asymmetric Aldol reaction. *Adv Synth Catal* 354(4):712–719
- Horning EC, Field RE (1946) Preparation of 3-methyl-5-aryl-2-cyclohexen-1-ones. *J Am Chem Soc* 68(3):384–387
- Horning EC, Denekas MO, Field RE (1944) The preparation of 4-carbethoxy-3-methyl-5-alkyl-2-cyclohexen-1-ones and 3-methyl-5-alkyl-2-cyclohexen-1-ones. *J Org Chem* 9(6):547–551
- Kazlauskas RJ (2005) Enhancing catalytic promiscuity for biocatalysis. *Curr Opin Chem Biol* 9(2):195–201
- Khersonsky O, Tawfik DS (2010) Enzyme promiscuity: a mechanistic and evolutionary perspective. *Annu Rev Biophys Biomol* 79:471–505
- Kitazume T, Ikeya T, Murata K (1986) Synthesis of optically active trifluorinated compounds: asymmetric Michael addition with hydrolytic enzymes. *J Chem Soc Chem Comm* 17:1331–1333
- Li K, He T, Li C, Feng XW, Wang N, Yu XQ (2009) Lipase-catalysed direct Mannich reaction in water: utilization of biocatalytic promiscuity for C–C bond formation in a “one-pot” synthesis. *Green Chem* 11(6):777–779
- Lin XF, Xu JM, Zhang F, Liu BK, Wu Q (2007) Promiscuous zinc-dependent acylase-mediated carbon–carbon bond formation in organic media. *Chem Commun* 20:2078–2080
- Lin XF, Wu Q, Xu JM, Li X, Wang JL (2009) Promiscuous zinc-dependent acylase-mediated one-pot synthesis of monosaccharide-containing pyrimidine derivatives in organic medium. *Adv Synth Catal* 351(11–12):1833–1841
- Liu ZQ, Xiang ZW, Wu Q, Lin XF (2013) Unexpected three-component domino synthesis of pyridin-2-ones catalyzed by promiscuous acylase in non-aqueous solvent. *Biochimie* 95(7):1462–1465
- Lou FW, Liu BK, Wu Q, Lv DS, Lin XF (2008) Candida antarctica lipase B (CAL-B)-catalyzed carbon-sulfur bond addition and controllable selectivity in organic media. *Adv Synth Catal* 350(13):1959–1962
- Martínez R, Mendoza HM, Angeles E (1998) 5-Aryl-3-methyl-2-cyclohexen-1-ones from 4-aryl-1, 4-dihydropyridines (Hantzsch esters). *Synth Commun* 28(15):2813–2820
- Mori K, Tamada S, Uchida M, Mizumachi N, Tachibana Y, Matsui M (1978) Synthesis of optically active forms of seudenol, the pheromone of douglas fir beetle. *Tetrahedron* 34(13):1901–1905
- Nakayachi T, Yasumoto E, Nakano K, Morshed S, Hashimoto K, Kikuchi H, Nishikawa H, Kawase M, Sakagami H (2004) Structure-activity relationships of α,β -unsaturated ketones as assessed by their cytotoxicity against oral tumor cells. *Anticancer Res* 24(2B):737–742
- Plummer EL, Stewart TE, Byrne K, Pearce GT, Silverstein RM (1976) Determination of the enantiomeric composition of several insect pheromone alcohols. *J Chem Ecol* 2(3):307–331
- Pollard DJ, Woodley JM (2007) Biocatalysis for pharmaceutical intermediates: the future is now. *Trends Biotechnol* 25(2):66–73

- Pollini GP, Benetti S, De Risi C, Zanirato V (2010) Hagemann's ester: a timeless building block for natural product synthesis. *Tetrahedron* 66(15):2775–2802
- Renard G, Lerner DA (2007) First simple and mild synthesis of 2-alkylbenzimidazoles involving a supported enzymatic catalyst. *New J Chem* 31(8):1417–1420
- Ross DW, Gibson KE, Thier RW, Munson AS (1996) Optimal dose of an antiaggregation pheromone (3-methylcyclohex-2-en-1-one) for protecting live douglas-fir from attack by *dendroctonus pseudotsugae* (Coleoptera: Scolytidae). *J Econ Entomol* 89(5):1204–1207
- Schmid A, Dordick JS, Hauer B, Kiener A, Wubbolts M, Witholt B (2001) Industrial biocatalysis today and tomorrow. *Nature* 409(6817):258–268
- Smith WT, Eftax DSP (1956) Analogs of “piperonyl cyclonene”. *J Org Chem* 21(2):174–176
- Svedendahl M, Hult K, Berglund P (2005) Fast carbon–carbon bond formation by a promiscuous lipase. *J Am Chem Soc* 127(51):17988–17989
- Torre O, Alfonso I, Gotor V (2004) Lipase catalysed Michael addition of secondary amines to acrylonitrile. *Chem Commun* 15:1724–1725
- Vité JP, Pitman GB, Fentiman AF Jr, Kinzer GW (1972) 3-Methyl-2-cyclohexen-1-ol isolated from *dendroctonus*. *Naturwissenschaften* 59(10):469
- Wang JL, Xu JM, Wu Q, Lv DS, Lin XF (2009) Promiscuous enzyme-catalyzed regioselective Michael addition of purine derivatives to α,β -unsaturated carbonyl compounds in organic solvent. *Tetrahedron* 65(12):2531–2536
- Wang L, Li C, Wang N, Li K, Chen X, Yu XQ (2010) Enzyme-mediated domino synthesis of 2-alkylbenzimidazoles in solvent-free system: a green route to heterocyclic compound. *J Mol Catal B Enzym* 67(1–2):16–20
- Wang JL, Liu BK, Yin C, Wu Q, Lin XF (2011a) *Candida antarctica* lipase B-catalyzed the unprecedented three-component Hantzsch-type reaction of aldehyde with acetamide and 1,3-dicarbonyl compounds in non-aqueous solvent. *Tetrahedron* 67(14):2689–2692
- Wang L, Gong QP, Liu XJ, Li YH, Huang P, Wang BQ, Zhao KQ (2011b) Organocatalyst-mediated Aldol-robinson cascade reactions: a convenient synthesis of substituted cyclohex-2-enones. *Chem Lett* 40(2):138–139
- Wu WB, Wang N, Xu JM, Wu Q, Lin XF (2005) Penicillin G acylase catalyzed Markovnikov addition of allopurinol to vinyl ester. *Chem Commun* 18:2348–2350
- Wu WB, Xu JM, Wu Q, Lv DH, Lin XF (2006) Promiscuous acylases-catalyzed markovnikov addition of *N*-heterocycles to vinyl esters in organic media. *Adv Synth Catal* 348(4–5):487–492
- Wu Q, Liu BK, Lin XF (2010) Enzymatic promiscuity for organic synthesis and cascade process. *Curr Org Chem* 14(17):1966–1988
- Wu MY, Li K, He T, Feng XW, Wang N, Wang XY, Yu XQ (2011) A novel enzymatic tandem process: utilization of biocatalytic promiscuity for high stereoselective synthesis of 5-hydroxyimino-4,5-dihydrofurans. *Tetrahedron* 67(14):2681–2688
- Xiang ZW, Liu ZQ, Liang YR, Wu Q, Lin XF (2013) L-Lysine/imidazole-catalyzed multicomponent cascade reaction: facile synthesis of C5-substituted 3-methylcyclohex-2-enones. *Chin J Chem* 31(8):997–1002
- Xu JM, Zhang F, Liu BK, Wu Q, Lin XF (2007) Promiscuous zinc-dependent acylase-mediated carbon–carbon bond formation in organic media. *Chem Commun* 20:2078–2080
- Zhou J, Wakchaure V, Kraft P, List B (2008) Primary-amine-catalyzed enantioselective intramolecular aldolizations. *Angew Chem Int Ed* 47(40):7656–7658
- Zhou LH, Wang N, Zhang W, Xie ZB, Yu XQ (2013) Catalytical promiscuity of α -amylase: synthesis of 3-substituted 2H-chromene derivatives via biocatalytic domino oxa-Michael/aldol condensations. *J Mol Catal B Enzym* 91:37–43