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Research paper

Enzymatic enantioselective aldol reactions of isatin derivatives with cyclic ketones under solvent-free conditions

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ABSTRACT

Nuclease p1 from *Penicillium citrinum* was observed to directly catalyze the asymmetric aldol reactions between isatin derivatives and cyclic ketones with high isolated yields (up to 95%) and moderate to good stereoselectivity (*dr* up to >99/1, *ee* up to 82%). A series of reaction conditions were investigated in detail, and the addition of deionized water had a big influence upon the enzyme activity. This case of biocatalytic promiscuity not only widens the applicability of nuclease p1 to new chemical transformation in organic synthesis, but also provides a potentially valuable method to construct pharmaceutically active compounds in medicinal chemistry.

1. Introduction

Since Klibanov pioneered the anhydrous enzymology in the early 1980s [1], enzymes as efficient and green biocatalysts in organic chemistry have gained much attention from chemists and biochemists due to their high selectivity, mild reaction conditions, environmental acceptability and potentially regenerable catalysts [2–6]. Especially, many enzymes have been discovered to catalyze unnatural reaction completely different from the originally performed, which is called biocatalytic promiscuity [7–11]. In the last decades, several elegant works on the enzymatic promiscuity have been reported, such as Michael addition [12–15], Henry reaction [16,17], Mannich reaction [18–20] and Markovnikov additions [21,22]. It is still in great demand and interest to develop novel activities of commercially available enzymes, particularly for the synthesis of bioactive molecules.

The asymmetric aldol reaction emerges as a simple and important method to form C–C bond in stereocontrolled organic chemistry. In 1974, Hajos and Parrish first described the organocatalyzed aldol reaction [23], and Shibasaki group reported the direct asymmetric aldol reaction of aldehydes with unmodified ketones in 1997 [24]. In the following decades, the aldehyde–aldehyde and aldehyde-ketone aldol reaction had been extensively researched [25–33], more recently some direct enzymatic asymmetric aldol reactions between aromatic aldehydes and ketones have been reported by Guan and Wang groups [34–38], however, the intermolecular ketone–ketone asymmetric aldol addition is rare and remains a significant challenge [39–41]. Considering that 3-substituted-3-hydroxyindolin-2-ones constitute a nucleus of a large variety of natural products and pharmaceutical candidates [42–47], such as compounds **A**, **B**, **C** and **D** in Fig. 1, a few reports about the aldol reactions between isatins and ketones had been published, but the catalysts were inevitably a base, an organic molecule and a metal complex [48–52]. In view of green chemistry, herein we wish to report a concise and straightforward approach to synthesize 3-alkyl-3-hydroxyindolin-2-ones by eco-friendly enzymes under solvent-free conditions. To the best of our knowledge, this is the first enzymatic asymmetric aldol reactions of isatins and cyclic ketones.

2. Material and methods

2.1. General information for reagents and analytical methods

Amano Lipase M from *Mucor javanicus* (≥10000 U/g enzyme activity, pH 7.0, 40 °C) was purchased from Sigma (Steinheim, Germany). Acidic protease from *Aspergillus usamii* No 537 (50 U/mg) and alkaline protease from *Bacillus licheniformis* No 2709 (200 U/mg) were purchased from Xuemei Enzyme Co. Ltd. (Wuxi, China). Chymopapain isolated from the latex of the unripe fruits of *Carica papaya* (20 U/mg, one unit of activity was defined as the amount of enzyme to produce TCA-soluble hydrolyzed products from casein, which gives an absorbance value equivalent to 1.0 μg of tyrosine at 275 nm/min at 30 °C and pH 7.5) and Nuclease P1 from *Penicillium citrinum* (5 U/mg.

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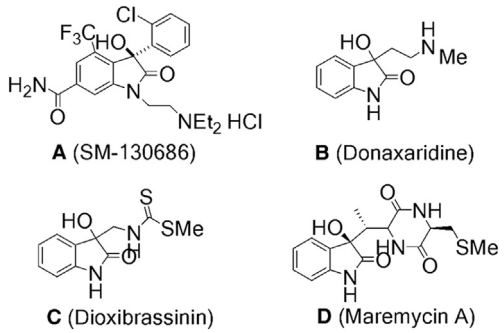


Fig. 1. Bioactive compounds with 3-substituted-3-hydroxyindolin-2-ones skeleton.

The activity was measured in terms of the amount of acid-soluble nucleotides produced by RNA hydrolysis which is catalyzed by nuclease p1. One unit of enzyme activity was defined as the amount of enzyme that produced an increase in the optical density of 1.0 in 1 min at 260 nm) were purchased from Guangxi Nanning Pangbo Biological Engineering Co. Ltd. (Nanning, China). Unless otherwise noted, all reagents were obtained from commercial suppliers and were used without further purification. The ^{1}H 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. The HPLC analysis was performed on SHIMADZU LC-20AT system at 254 nm.

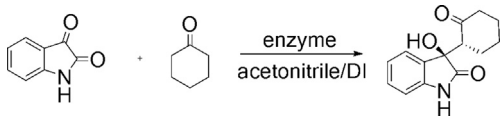
2.2. General procedure for the synthesis of racemic products between isatin derivatives and cyclic ketones

To a stirred solution of 2 mmol isatin derivative and 3 mmol cyclic ketone in 20 mL methanol was added 5–6 drop diethylamine and the mixture was left to react for about 4–6 h at room temperature until isatin derivative disappeared, determined by TLC. Then the solution was concentrated *in vacuo* and the obtained mixture was washed in boiling petrol ether/ethyl acetate, recrystallized in them.

2.3. General procedure for the enzymatic aldol reactions of isatins and cyclic ketones

To a solution of isatin derivative (0.1 or 0.2 mmol) and DI (0.15 mL) in cyclic ketone (1 mL) was added nuclease p1 (150 mg), the flask was placed in incubator shaker at 18 °C for 120 h at 200 r/min. When the reaction was finished, the organic phase was purified by column chromatography with petroleum ether/ethyl acetate as eluent. Or the products were obtained after solvent in organic phase volatilized thoroughly.

Table 1
Screening of enzymes on the aldol reaction.[a]



Entry	Enzyme	Yield (%)[b]	*dr*[c]	*ee* (%)[d]
1	Chymopapain	57	92/8	37
2	MJL	99	94/6	29
3	Nuclease p1	15	87/13	56
4	AUAP	54	90/10	32
5	BLAP	75	90/10	12

[a] Conditions: isatin (0.2 mmol), cyclohexanone (1 mmol), acetonitrile (1 mL), DI (0.1 mL), enzyme (70 mg), 30 °C, 120 h.
[b] Determined by chiral HPLC.
[c] The *anti*/*syn* ratio, determined by chiral HPLC analysis of the diastereomeric isomers.
[d] The *anti* enantiomeric excess, determined by chiral HPLC analysis, absolute configurations assigned by comparison with literature data [48,53,54].

3. Results and discussion

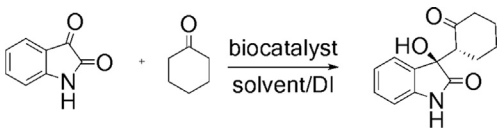
3.1. The catalytic activity of different enzymes for the asymmetric aldol reactions of isatins and cyclic ketones

Initial experiments of isatin and cyclohexanone catalyzed by different enzymes were performed in acetonitrile with 0.1 mL deionized water (DI). We were delighted to see the enantioselectivity of enzymes for the aldol reaction. The data are summarized in Table 1, the results indicated that the activity and enantioselectivity of varied biocatalysts were contradictory. For example, Amano Lipase M from *Mucor javanicus* (MJL) exhibited excellent activity with 99% yield, but poor enantioselectivity (Table 1, entry 3), on the contrary, the best enantioselectivity of 56% *ee* value was obtained by nuclease p1 from *P. citrinum* (nuclease p1) under the same conditions as MJL, but the yield is poor (Table 1, entry 4). Other proteinase, such as Chymopapain, Acidic protease from *A. usamii* (AUAP) and alkaline protease from *B. licheniformis* (BLAP), showed lower enantioselectivity than nuclease p1 (Table 1, entries 1, 4 and 5). The size of active center in different enzymes varied a lot, substrate specificity was understandable here. Based on the importance of enantioselectivity, nuclease p1 was chosen as the biocatalyst for the following optimization.

3.2. The effect of reaction media on the asymmetric aldol reactions of isatins and cyclic ketones

Considering the reaction medium has a great effect on the activity and the stability of an enzyme, different solvents were screened for the asymmetric aldol reactions of isatins and cyclic ketones. Data in Table 2 showed the catalytic activity and stereoselectivity were obviously influenced by the reaction media. Nuclease p1 displayed the best enantioselectivity in acetonitrile

Table 2
Solvent screening and controlled experiments.[a]



Entry	Solvent	Yield (%)[b]	*dr*[c]	*ee* (%)[d]
1	DMSO	96	92/8	5
2	DMF	99	96/4	5
3	EG	60	79/21	16
4	Dioxane	33	72/28	34
5	Acetonitrile	15	87/13	56
6	Toluene	–	–	–
7	THF	–	–	–
8	Acetonitrile[e]	–	–	–
9	Acetonitrile[f]	–	–	–

[a] Conditions: isatin (0.2 mmol), cyclohexanone (1 mmol), acetonitrile (1 mL), DI (0.1 mL), enzyme (70 mg), 30 °C, 120 h.
[b] Determined by chiral HPLC.
[c] The *anti*/*syn* ratio, determined by chiral HPLC analysis of the diastereomeric isomers.
[d] The *anti* enantiomeric excess, determined by chiral HPLC analysis.
[e] Denatured with EDTA at 100 °C for 24 h.
[f] No enzyme.

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