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Graphical Abstract

Synthesis of 2,3-dihydroquinazolin-4(1*H*)-ones catalyzed by *α*-chymotrypsin

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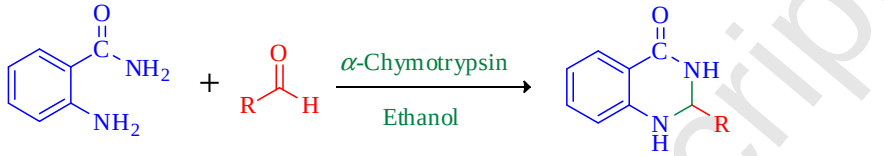

α-Chymotrypsin has a promiscuous ability to catalyze the cyclocondensation of aldehydes with 2-aminobenzamides to afford the corresponding 2,3-dihydroquinazolin-4(1*H*)-ones.

Original article

Synthesis of 2,3-dihydroquinazolin-4(1*H*)-ones catalyzed by α-chymotrypsin

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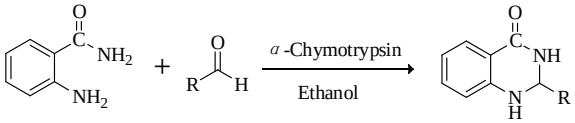
ABSTRACT

We discovered that *α*-chymotrypsin has a promiscuous ability to catalyze the cyclocondensation of aromatic and aliphatic aldehydes with 2-aminobenzamides to afford the corresponding 2,3-dihydroquinazolin-4(1*H*)-ones successfully in high yields (89%-98%) under alcohol solvent. The catalytic activity of *α*-chymotrypsin was evaluated through investigating the temperature, the enzyme loading and the ratio of substrates in the enzyme-catalyzed reactions. The present method proves to be efficient and environmentally friendly in terms of short reaction time, high yield, green catalyst and the clean products obtained without further purification processes.

1. Introduction

2,3-Dihydroquinazolin-4(1*H*)-one derivatives acquired significance due to their broad range of potential biological and pharmacological activities, as well as their crucial role in the preparation of drug molecules and natural products. 2,3-Dihydroquinazolinone derivatives act as important intermediates that can be easily oxidized to their quinazolin-4(3*H*)-one analogues, which also include important biologically active compounds. In recent years, the protocols for the synthesis of 2,3-dihydroquinazolin-4(1*H*)-ones have been developed in different ways using iodine [1], succinimide-*N*-sulfonic acid [2], *p*-TSA [3], 2-morpholinoethanesulfonic acid [4], $ZrCl_4$ [5], NH_4Cl [6], $KAl(SO_4)_2$ [7], silica sulfuric acid [8], sulfamic acid [9], nanocrystalline sulfated zirconia [10], *N*-propylsulfamic acid on magnetic nanoparticles [11], metal-CNTs [12], *β*-cyclodextrin-SO_3H [13], PEG-400 [14] and ionic liquids [15] as catalysts. These synthetic methodologies are useful to facilitate the synthesis of the desired compounds in many instances. However some of the synthetic strategies have certain limitations such as tedious processes, long reaction time, low yields of product, expensive moisture-sensitive catalysts, cumbersome preparation processes for the required catalyst, and liberating hazardous HF during recycling. Therefore, there has been increasing effort on the design and use of environmentally compatible catalysts for the preparation of 2,3-dihydroquinazolin-4(1*H*)-one derivatives owing to the growing demand for the development of more sustainable and environmentally-friendly processes.

Enzymes have received great attention as sustainable, efficient, green and biodegradable catalysts for the synthesis of pharmaceutical, industrial and agricultural chemicals and intermediates. Among the promiscuous enzymes, hydrolases beyond doubt play an important role because of their high stability, availability, and broad range of substrates. Recently, several enzymes have been used in multiple types of organic reactions such as asymmetric aldol reactions [16], asymmetric Michael additions [17] and asymmetric Henry reactions [18]. However, to the best of our knowledge, enzyme-catalyzed cyclocondensation reaction for the preparation of 2,3-dihydroquinazolin-4(1*H*)-one derivatives has never been reported. Herein, we wish to report the first discovery that *α*-chymotrypsin could catalyze the reaction of 2-aminobenzamide with a series of aldehydes to afford the corresponding 2,3-dihydroquinazolinones in ethanol (Scheme 1), and the yields up to 98% were achieved, which was obviously a novel environmentally benign synthetic method.



Scheme 1. The *α*-chymotrypsin-catalyzed cyclocondensation of 2-aminobenzamide with aldehydes.

2. Experimental

2.1. General

Alkaline protease (200 U/mg enzyme activity) and Papain (800 U/mg enzyme activity) were purchased from Jiangsu Ruiyang Biotechnology Co., Ltd. (Jiangsu, PR China). Trypsin from bovin pancreas (≥ 2500 U/mg enzyme activity), *α*-chymotrypsin (800 U/mg

enzyme activity) and albumin from bovine serum were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, PR China). Protease from *Aspergillus saitoi* (≥ 0.6 U/mg enzyme activity), proteinase from *Aspergillus melleus* (3.3 U/mg enzyme activity) and protease from *Bacillus licheniformis* (2.4 U/g enzyme activity) were purchased from Sigma Aldrich Co., USA. All reagents were obtained from commercial suppliers and used without further purification except that 2-aminobenzamide was recrystallized. Melting points were obtained on a WRS-1B Digital Melting Point Apparatus. The NMR spectra were recorded on a Bruker 400 MHz instrument using DMSO-d_6 as a solvent. Chemical shifts (δ) were expressed in ppm using tetramethylsilane (TMS) as an internal standard, and coupling constants (J) were reported in Hz.

2.2 General procedure for the synthesis of 2,3-dihydroquinazilin-4(1H)-ones

α-Chymotrypsin (2 mg/mL) was added to a solution of 2-aminobenzamide (1 mmol) and aldehyde (1.8 mmol) in ethanol (10 mL) and the mixture was stirred at 60 ºC in a constant temperature incubator shaker for a specified period of time. After the completion of the reaction, water (20 mL) was added to the mixture to quench the reaction, the enzymes and the excess aldehydes were dissolved in an aqueous ethanol solution, and the desired products precipitated out. The products were collected by filtration without further purification.

3. Results and discussion

Based on our previous research, initial efforts were undertaken using 2-aminobenzamide and 4-methylbenzaldehyde as a model reaction, and ethanol was chosen as the reaction medium. In order to select the most efficient catalyst for the cyclocondensation, eight proteases were screened using the model system. As shown in Table 1, the best yield of 62% was achieved using α-chymotrypsin (Table 1, entry 8) while trypsin from bovin pancreas showed low activity in this reaction and only provided 13% yield (Table 1, entry 2). However, the other tested enzymes demonstrated no catalytic ability. Non-enzyme protein BSA (Bovine serum albumin) (Table 1, entry 9) and denatured α-chymotrypsin (Table 1, entry 10) were also used as controls to demonstrate the specific catalytic effect of the α-chymotrypsin, and both barely showed catalytic activity, producing the similar results as the blank control reaction (Table 1, entry 11). All the results clearly indicated that the catalytic ability of the α-chymotrypsin is responsible for the model reaction.

Table 1
The catalytic activities of different enzymes.[a]

Entry	Enzymes	Yield (%)[b]
1	Alkaline protease	5
2	Trypsin	13
3	Papain	9
4	Proteinase from *Aspergillus melleus*	7
5	Papain from papayalatex	6
6	Protease from *Aspergillus saitoi*	6
7	Protease from *Bacillus licheniformis*	7
8	α-Chymotrypsin	62
9	BSA	6
10	Denatured α-chymotrypsin[c]	6
11	No enzyme	5

[a] Reaction conditions: 2-aminobenzamide 1 mmol, 4-methylbenzaldehyde 1 mmol, enzyme 2.0 mg/mL and ethanol 10 mL, stirred at 40 ºC for 60 min.
[b] Isolated yield.
[c] Pretreated with urea solution (8 mol/L).

To improve the catalytic ability of the enzyme, we carried out some experiments focusing on the effect of reaction temperature, which plays a significant role in maintaining the stability and catalytic activity of enzymes. The range of temperature from 30 ºC to 70 ºC was screened for the α-chymotrypsin-catalyzed cyclocondensation reactions and the results were shown in Table 2, entries 1-4. The best yield of 89% was achieved at 60 ºC (Table 2, entry 3) and higher temperature (70 ºC) gave lower yield probably due to the partial denaturation of the enzyme at higher temperatures. So, we chose 60 ºC as an optimal temperature for the reaction. Next, the effect of enzyme loading on the α-chymotrypsin-catalyzed model reaction was investigated in order to further optimize the reaction conditions. As shown in Table 2, entries 5-9, the model reaction only gave the product in a low yield of 5% in the absence of enzyme. However, an obvious increase in the yield (77%) was detected when 0.5 mg/mL of α-chymotrypsin was added. After that, only a slight rising trend was observed with the larger catalyst dosage from 0.5 mg/mL to 2.5 mg/mL, thus, 2.0 mg/mL was chosen as the best enzyme loading.

Table 2
The effect of temperature and enzyme loading on the model reaction.[a]

Entry	Temperature (ºC)	Enzyme loading (mg/mL)	Yield (%)[b]
1	30	2.0	15
2	50	2.0	76
3	60	2.0	89
4	70	2.0	85
5	60	0	5
6	60	0.5	77
7	60	1.0	83
8	60	1.5	85
9	60	2.5	88

[a] Reaction conditions: 2-aminobenzamide 1 mmol, 4-methylbenzaldehyde 1 mmol, α-chymotrypsin and ethanol 10 mL for 60 min.

[b] Isolated yield.

Some further experiments were performed to investigate the effects of the molar ratio of substrates on the *α*-chymotrypsin catalyzed model reaction. As shown in Table 3, increasing the molar ratio of 2-aminobenzamide to 4-methylbenzaldehyde from 1.0 : 1.2 to 1.0 : 1.8 led to an growing yield (from 89% to 98%) of the desired product and further increasing the molar ratio from 1.0 : 1.8 to 1.0 : 2.0 hardly improved the results. Thus, we chose the molar ratio of 2-aminobenzamide to 4-methylbenzaldehyde of 1.0 : 1.8 as the optimal proportion of the substrate for the reaction.

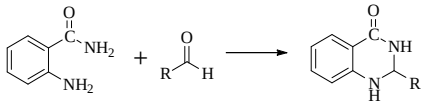
Table 3
The effect of molar ratio on the model reaction.[a]

Entry	Molarratio[b]	Yield (%)[c]
1	1:1.2	89
2	1:1.4	92
3	1:1.6	95
4	1:1.8	98
5	1:2.0	98

[a] Reaction conditions: α-chymotrypsin 2.0 mg / mL and ethanol 10 mL, stirred at 60ºC for 30 min.
[b] 2-Aminobenzamide / 4-methylbenzaldehyde (mmol / mmol).
[c] Isolated yield.

Finally, we further investigated the substrate scope and the limitation of the *α*-chymotrypsin-catalyzed cyclocondensation reaction by employing a wide range of aldehydes under the optimized conditions. The results are summarized in Table 4. It can be seen that the corresponding dihydroquinazolin-4(1*H*)-ones were obtained with good to excellent yields in all cases. Furthermore, various types of aromatic aldehydes with electron-donating groups reacted very well with 2-aminobenzamide under the optimized reaction conditions, as well as aromatic aldehydes containing electron-withdrawing substituents, such as the methyl- and bromo-substituted benzaldehydes afforded the corresponding products **b** and **f** in 98% and 96% yields, respectively. Generally, the reaction could proceed smoothly and was not influenced by substituents in the aromatic ring. To our delight, aliphatic aldehydes such as caprylaldehyde were compatible with the reaction conditions to give the desired product (**o**) in high yield of 95%. And hexaldehyde also afforded the corresponding product (**n**) in excellent yield although another 30 minutes of reaction time is required.

Table 4
Investigation of the reactant scope of the cyclocondensation reaction for synthesis of 2,3-dihydroquinazolin-4(1*H*)-one derivatives.[a]



Entry	R	Time (min)	Compd	Yield (%)[b]	MP (°C) Found	MP (°C) Lit. (ref.)
1	C_6H_5	30	**a**	91	225-226	224-226 [4]
2	4-CH_3-C_6H_4	30	**b**	98	227-228	225-227 [12]
3	3-CH_3-C_6H_4	30	**c**	96	204-205	
4	4-Cl-C_6H_4	30	**d**	96	202-203	205-207 [19]
5	2- Cl-C_6H_4	30	**e**	98	208-209	207-209 [19]
6	3-Br-C_6H_4	30	**f**	96	190-191	227-228 [19]
7	2-Br-C_6H_4	50	**g**	98	171-182	172-173 [12]
8	4-OCH_3-C_6H_4	30	**h**	92	188-189	181-183 [12]
9	3-OH-C_6H_4	30	**i**	95	206-207	215-217 [20]
10	2-OH-C_6H_4	30	**j**	96	223-225	220-221 [4]
11	4-OH-3OMe-C_6H_4	30	**k**	97	224-225	227-228 [4]
12	$(CH)_2$-C_6H_4	30	**l**	91	173-175	155-157 [20]
13	C_5H_{11}	60	**m**	90	154-155	160-162 [20]
14	C_7H_{15}	30	**n**	98	158-159	

[a] Reaction conditions: 2-aminobenzamide 1 mmol, aldehyde 1.8 mmol, *α*-chymotrypsin 2.0 mg / mL, ethanol 10 mL, stirred at 60 ºC.
[b] Isolated yield.

4. Conclusion

In summary, we have succeeded in obtaining dihydroquinazolin-4(1*H*)-ones using *α*-chymotrypsin as a new biocatalyst. A wide range of aromatic aldehydes and aliphatic aldehydes could be used to give the products in excellent yields. Notably, The unnatural ability of *α*-chymotrypsin to catalyze a cyclocondensation reaction to prepare 2,3-dihydroquinazolin-4(1*H*)-one derivatives was discovered for the first time. Compared with current chemical approaches, this enzymatic method is more environmental-friendly and sustainable using a biocatalyst from bio-degradable and inexpensive sources. Moreover, the catalyst *α*-chymotrypsin accommodated broad substrates and produced the desired products in high yields in a short period of time.

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