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Bovine serum albumin-catalyzed one-pot synthesis of 2-aminothiophenes via Gewald reaction

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ABSTRACT

A novel bovine serum albumin (BSA)-catalyzed Gewald reaction in one-pot was developed in this work. The influence of reaction conditions including solvent, temperature and catalyst loading was investigated, and 12 multi-substituted 2-aminothiophene derivatives were prepared with moderate to excellent yields. Recycle experiments were designed to demonstrate the reusability of BSA. This novel activity of BSA to catalyze Gewald reaction is of practical significance in expanding the application of biocatalysts.

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1. Introduction

Substituted 2-aminothiophenes are a kind of important structures which usually show diverse pharmacological profiles with antimicrobial, anticonvulsant and anti-inflammatory activities [1], and thus are continually used as a scaffold to construct a series of natural products, dyes and agrochemicals [2]. They can also serve as potential JNK2 and JNK3 kinase inhibitor and adenosine agonists [3]. Many other commercial medicines and drug candidates have been increasingly evolved from such scaffold as well [4]. The preparation of 2-aminothiophene derivatives is always achieved by a Gewald reaction (Scheme 1), which is a multicomponent condensation of a ketone with an activated α-hydrogen, a cyanomethylene containing an electron-withdrawing group, e.g., cyanoacetate, and elemental sulfur in the presence of an organic base [5] such as morpholine [6], diethylamine [7], etc. Alternative catalysts like L-proline [8] have also been developed for Gewald reaction. Yet, existing chemical procedures are always associated with all kinds of weaknesses, like high catalyst loading [9], complicated procedures [10], and hazardous solvents [11], which hinder the further development of the Gewald reaction. It is of special significance to investigate new catalysts in terms of environmental sustainability, operational simplicity, and broad substrate scope.

Since the past decade, dynamic efforts to exploit the biocatalysts, like protein (enzyme) [12], DNA [13], whole cell [14], etc. for catalyzing organic reactions have been on the raise due to their simple processing requirements and high selectivity. However, for the Gewald reaction, to the best of our knowledge, no biocatalytic procedures have been reported. This current situation and other relevant reports encourage us to explore the biocatalyzed Gewald reaction. Among all the catalysts tested, bovine serum albumin (BSA) is a powerful one which has been reported with many kinds of catalytic activity. In 2011, Gotor and co-workers reported that BSA could efficiently promote the nitroaldol addition between aromatic aldehydes and 1-nitroal-kanes in aqueous medium [15]; stereoselective thio-Michael addition to chalcones in water catalyzed by BSA was reported by Gaggero in 2011 [16]; BSA could also serve as the catalyst for the one-pot synthesis of benzimidazoles and aldehydes [17]. First bovine serum albumin-promoted synthesis of enones, cinnamic acids and coumarins in ionic liquid and bovine serum albumin-triggered waste-free synthesis of 3,4-dihydropyrimidin-2-(1H)-ones were also developed [18]. In this work, the Gewald reaction could be catalyzed by BSA with satisfying yields.

2. Materials and methods

2.1. Materials

D-Aminoacylase from *Escherichia coli* (10,000 U/mg, 1 U is defined as enzyme quantity which produces 1 μmol of D-amino acid per 30 min) and Acylase "Amano" (AA) from *Aspergillus oryzae*

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$R_1C(=O)CH_2R_2$ + GWE–CH₂–CN + S_8 → 3

Scheme 1. Gewald reaction.

(≥30,000 U/g, 1 U is defined as enzyme quantity which produces 1 μmol of L-amino acid per 30 min) were purchased from Amano Enzyme Inc. (Japan). Lipase from *Hog pancreas* (2.4 U/mg, 1 U is the amount of immobilized enzyme which forms 1% octyl laurate from 0.5 mmol lauric acid and 1.0 mmol 1-octanol in 10 mL water-saturated isooctane in 1 h at 20 °C), Amano Lipase M from *Mucor javanicus* (≥10,000 U/g enzyme activity, pH 7.0, 40 °C), Lipase immobilized on acrylic resin from *Candida antarctica* (≥10,000 U/g, recombinant, expressed in *A. oryzae*) and lipase from *Porcine pancreas* (30–90 U/mg protein, 1 U will hydrolyze 1.0 mequiv. of triacetin in 1 h at pH 7.7 at 37 °C) were purchased from Sigma (Steinheim, Germany). Lipase AY 30 (700–1500 U/mg solid, 1 U will hydrolyze 1.0 mequiv. of olive oil from a triglyceride in 1 h at pH 7.7 at 37 °C) was purchased from Acros (New Jersey, USA). Lipozyme immobilized from *Mucor miehei* (MML) was purchased from Fluka. Bovine serum albumin (BSA) was obtained from Wuxi Enzyme Co. Ltd., Wuxi, PR China. L-Glutamic acid, L-threonine, L-serine, L-cysteine and L-lysine were obtained from Sinopharm Chemical Reagent Co., Ltd. All other chemicals were of the highest purity commercially available.

2.2. General procedure for the synthesis of 2-aminothiophenes

A mixture of **1** (1 mmol), **2** (1 mmol), elemental sulfur (1 mmol) and BSA (20 mg) was added to 1 mL of DMF. The reaction was incubated at 50 °C and 200 rpm. After the required time, the BSA was filtered off to terminate the reaction. For the products with high yields, the solid crude products precipitated in water, and then followed by filtration and drying. For the products with low yields, the crude residues were purified by flash column chromatography on silica gel using petroleum/ethyl acetate.

The structures of the products were confirmed by IR, ^{1}H NMR and ^{13}C NMR. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 spectrometer in $CDCl_3$ using TMS (tetramethylsilane) as internal reference. IR spectra were obtained with a Nicolet Nexus 470 FT-IR spectrophotometer. HPLC was carried out using an Agilent 1100 series with an Agilent TC-C18 column (**3a**, **3c**, **3e**, **3g**, **3i**, **3k**: methanol/water ratio = 60/40, 1.0 mL/min and 220 nm; **3b**, **3d**, **3f**, **3h**, **3g**, **3l**: methanol/water ratio = 60/40, 1.0 mL/min and 229 nm).

For all reactions, solvents for column chromatography were distilled before use.

2.2.1. 2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (*3a*)

Yellow solid, mp 140–142 °C (lit. [19] mp 144–146 °C). IR (KBr): ν. 3447, 3329, 2198 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 4.62 (bs, 2H), 2.53–2.46 (m, 4H), 1.85–1.73 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 159.9, 132.4, 120.7, 115.5, 88.8, 24.5, 24.1, 23.4, 22.1.

2.2.2. Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (*3b*)

Yellow solid, mp 114–115 °C (lit. [19] mp 117–118 °C). IR (KBr): ν. 3403, 3299, 1647 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ: 5.93 (bs, 2H), 4.25 (q, J = 7.2 Hz, 2H), 2.72–2.68 (m, 2H), 2.52–2.46 (m, 2H), 1.82–1.70 (m, 4H), 1.33 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 166.1, 161.2, 132.5, 118.0, 106.1, 59.4, 26.9, 24.6, 23.3, 22.8, 14.5.

2.2.3. 2-Amino-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carbonitrile (*3c*)

Yellow solid. mp 152–153 °C (lit. [20] mp 151 °C). IR (KBr): ν. 3438, 3336, 2194 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 4.64 (bs, 2H), 2.78–2.66 (m, 4H), 2.41–2.31 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 166.2, 142.9, 125.3, 115.5, 85.6, 29.3, 28.5, 27.4.

2.2.4. Ethyl 2-amino-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxylate (*3d*)

Yellow solid. mp 181–182 °C (lit. [21] mp 182–183 °C). IR (KBr): ν. 3413, 3297, 1653 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 5.85 (bs, 2H), 4.24 (q, J = 6.8 Hz, 2H), 2.85–2.78 (m, 2H), 2.75–2.67 (m, 2H), 2.36–2.25 (m, 2H), 1.32 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 166.2, 165.7, 143.1, 122.1, 102.7, 59.5, 30.8, 28.9, 27.2, 14.4.

2.2.5. 2-Amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carbonitrile (*3e*)

Yellow solid. mp 125–126 °C (lit. [20] mp 126 °C). IR (KBr): ν. 3443, 3310, 2203 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ: 4.50 (bs, 2H), 2.64–2.54 (m, 4H), 1.85–1.77 (m, 2H), 1.68–1.60 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 158.1, 136.9, 123.7, 115.9, 91.7, 31.9, 29.9, 29.4, 28.1, 27.2.

2.2.6. Ethyl 2-amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxylate (*3f*)

Yellow solid. mp 87–88 °C (lit. [20] mp 89 °C). IR (KBr): ν. 3397, 3301, 1652 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 5.76 (bs, 2H), 4.28 (q, J = 7.2 Hz, 2H), 2.97 (t, J = 5.6 Hz, 2H), 2.57 (t, J = 5.6 Hz, 2H), 1.85–1.76 (m, 2H), 1.68–1.56 (m, 4H), 1.34 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 165.9, 159.8, 138.0, 121.3, 107.6, 59.6, 32.1, 29.0, 28.7, 27.8, 26.9, 14.4.

2.2.7. 2-Amino-4-methylthiophene-3-carbonitrile (*3g*)

Yellow solid. mp 118 °C (lit. [22] mp 118–119 °C). IR (KBr): ν. 3419, 3325, 2204 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 5.96 (s, 1H), 4.75 (bs, 2H), 2.19 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 162.3, 135.8, 115.4, 105.2, 90.8, 15.3.

2.2.8. Ethyl 2-amino-4-methylthiophene-3-carboxylate (*3h*)

Yellow solid. mp 78 °C (lit. [23] mp 76–78 °C). IR (KBr): ν. 3411, 3301, 1651 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 6.03 (bs, 2H), 5.83 (s, 1H), 4.28 (q, J = 7.2 Hz, 2H), 2.28 (s, 3H), 1.35 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 172.7, 160.9, 127.3, 114.7, 104.0, 60.6, 20.1, 13.1.

2.2.9. 2-Amino-4,5-dimethylthiophene-3-carbonitrile (*3i*)

Yellow solid. mp 142 °C (lit. [24] mp 141–142 °C). IR (KBr): ν. 3432, 3335, 2200 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 4.58 (bs, 2H), 2.15 (s, 3H), 2.06 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 158.9, 129.7, 117.3, 115.3, 90.9, 22.71, 13.1.

2.2.10. Ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate (*3j*)

Yellow solid. mp 94 °C (lit. [24] mp 91–92 °C). IR (KBr): ν. 3403, 3303, 1648 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ: 5.89 (bs, 2H), 4.27 (q, J = 7.2 Hz, 2H), 2.16 (s, 3H), 2.15 (s, 3H), 1.35 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 166.1, 161.0, 130.4, 113.9, 107.7, 59.5, 14.8, 14.4, 12.3.

2.2.11. 2-Amino-4-ethyl-5-methylthiophene-3-carbonitrile (*3k*)

Yellow solid. mp 154 °C (lit. [25] mp 155–159 °C). IR (KBr): ν. 3430, 3321, 2199 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 4.60 (bs, 2H), 2.47 (q, J = 7.6 Hz, 2H), 2.16 (s, 3H), 1.14 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 159.6, 136.4, 118.4, 115.7, 89.1, 21.0, 14.3, 12.2.

Table 1
Optimization of reaction catalysts.[a]

O + NC⌒CN + S_8 —catalyst, DMF, 50 °C→ (CN, NH_2, S)

Entry	Catalyst	Reaction time (h)	Yield (%)[b]
1	–	12	Trace
2	Lipase from porcine pancreas	12	71
3	Lipase from hog pancreas	12	67
4	Lipozyme, MML	12	69
5	Alkaline protease from *Bacillus subtilis*	12	66
6	Acylase "Amano" from *Aspergillus oryzae*	12	68
7	Amano Lipase M from *Mucor javanicus*	12	64
8	Lipase AY 30	12	35
9	Lipase B acrylic resin from *Candida antarctica*	12	55
10	D-Aminoacylase from *Escherichia coli*	12	83
11	BSA	4	>99

[a] Reactions were performed with cyclohexanone (1 mmol), malononitrile (1 mmol), sulfur (1 mmol) and catalysts (20 mg) in DMF (1 mL) at 50 °C.
[b] All yields were determined by HPLC

2.2.12. *Ethyl 2-amino-5-phenylthiophene-3-carboxylate (**3l**)*

Yellow solid. mp 126 °C (lit. [26] mp 126 °C). IR (KBr): ν. 3455, 3323, 1668 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ: 7.44 (d, J = 7.6 Hz, 2H), 7.36–7.29 (m, 2H), 7.24 (s, 1H), 7.23–7.17 (m, 1H), 6.01 (bs, 2H), 4.30 (q, J = 7.2 Hz, 2H), 1.37 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 165.4, 161.9, 134.0, 128.8, 126.6, 125.0, 124.7, 121.2, 108.1, 59.9, 14.6.

3. Results and discussion

3.1. *Screening of different biocatalysts*

At first, different biocatalysts for the model reaction between cyclohexanone, propanedinitrile and elemental sulfur in DMF at 50 °C were screened. The screening results of the reaction were summarized in Table 1. Only a trace amount of the desired product was detected in the absence of the catalyst (entry 1, Table 1) after 12 h. When adding some enzymes, the yields increased significantly (entries 2–10, Table 1). Under the same condition, when BSA was used as the biocatalyst, the yield increased dramatically (entry 11, Table 1) during a much shorter reaction time. This is due to the rich variety of surface amino acids of BSA which make it much more worthy to be investigated. What is more, it could also be immobilized on other kinds of carriers to obtain a more recyclable version. Therefore, BSA was chosen for further optimization of reaction conditions.

3.2. *Influence of reaction media*

The reaction media usually shows great influence on the biocatalytic reactions. To gain a much deeper insight of this reaction, ten solvents with different polarities or other properties were chosen. The lowest activity was expressed in low polar solvents such as Hexane, Toluene and $CHCl_3$. In solvents like H_2O and *t*-BuOH, the reaction yields were less than 15% (entries 4 and 5, Table 2). Better results were obtained in THF or CH_3CN (entries 6 and 7, Table 2). In DMSO, EtOH and DMF, the yields were improved to 80%, 86% and 99% (entries 8, 9 and 10, Table 2), respectively after just 4 h. According to these results, DMF was chosen as the medium for further investigation.

Table 2
Optimization of solvent effect on the model reaction.[a]

Entry	Solvent	Yield (%)[b]
1	Hexane	trace
2	Toluene	trace
3	$CHCl_3$	trace
4	H_2O	10
5	*t*-BuOH	15
6	THF	30
7	CH_3CN	55
8	DMSO	80[c]
9	Ethanol	86[c]
10	DMF	>99[c]

[a] Reactions were performed with cyclohexanone (1 mmol), malononitrile (1 mmol), sulfur (1 mmol) and BSA (20 mg) in 1 mL mentioned solvent for 12 h.
[b] All yields were detected by HPLC.
[c] The reaction was carried out for 4 h.

3.3. *Optimization of reaction time*

Fig. 1 shows that the yield of the model reaction raised with the increase of reaction time. It reaches 99% after 4 h. However, there was no significant change in the reaction yield by further increasing the reaction time.

3.4. *Influence of temperature*

In most of the articles, it has been reported that the reaction temperature is an important parameter in biocatalysis. Therefore, four different temperatures in the range of 20–70 °C were selected for the reaction (Fig. 2). Within the examined temperature range between 20 and 50 °C, higher product yield was achieved along with the rise of the reaction temperature. However, a further increase in temperature led to a slight drop in the yield, which could be attributed to the partial inactivation of biomolecules at a higher temperature. Therefore, it is important to choose an appropriate operating temperature for maintaining the activity. It was observed that the yield of reaction reached its maximum at 50 °C, which was selected as the optimum temperature for the reaction.

3.5. *Influence of catalyst loading*

To select the most appropriate amount of BSA loading, a number of experiments were performed in the range from 0 to 25 mg/mL under similar conditions (Fig. 3). The reaction yield increased with

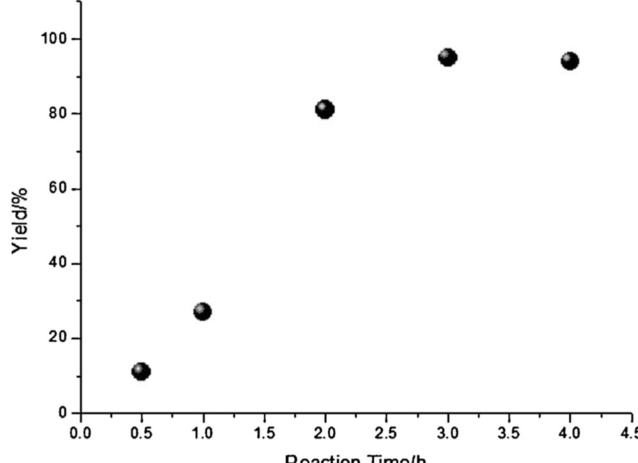

Fig. 1. Time-trend analysis of the model reaction catalyzed by BSA. Reactions were performed with cyclohexanone (1 mmol), malononitrile (1 mmol), sulfur (1 mmol) and BSA (20 mg) in DMF (1 mL).

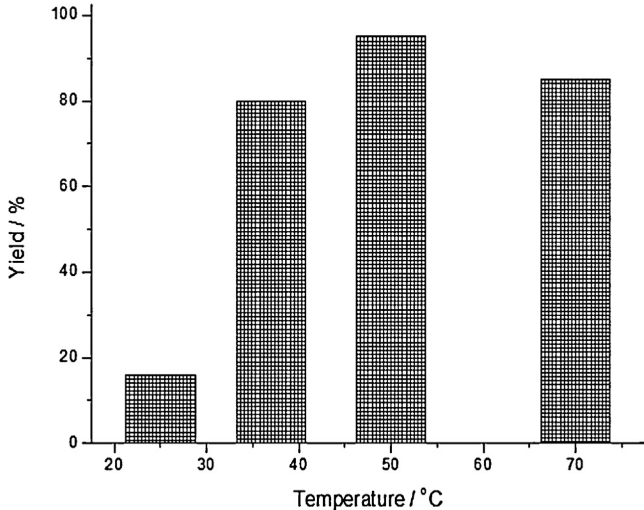

Fig. 2. Effect of temperature on the Gewald model reaction catalyzed by BSA. Reactions were performed with cyclohexanone (1 mmol), malononitrile (1 mmol), sulfur (1 mmol) and BSA (20 mg) in DMF (1 mL) for 4 h and all yields were determined by HPLC.

higher BSA loadings, up to 20 mg/mL. Above this loading value (25 mg/mL), there was no significant improvement in the yield of reaction. Thus further experiments were carried out at 20 mg/mL enzyme loading.

3.6. Catalyst reusability

Recyclability is an important factor for the use of biocatalysts. To investigate the reusability, BSA was washed with acetone for three times and then dried after every catalysis cycle and reintroduced into a fresh Gewald Reaction by adding another 2 mg of BSA. The effect of recycle usage on the reaction yield is shown in Fig. 4. It was observed that the stability of BSA after 5× usage still retained 90%.

3.7. Influence of substrate structure

The substrate scope of the BSA-catalyzed Gewald reaction was investigated starting from a series of activated nitriles and elemental sulfur in one pot under the optimized conditions as mentioned above. As shown in Table 3, the BSA-catalyzed Gewald reaction

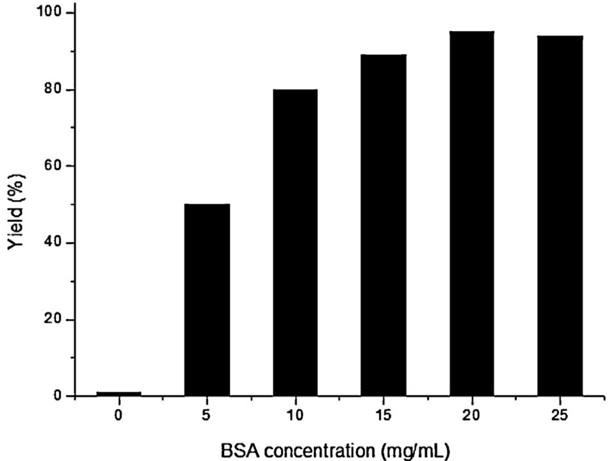

Fig. 3. Effect of BSA concentration on the model reaction. Reactions were performed with cyclohexanone (1 mmol), malononitrile (1 mmol), sulfur (1 mmol) and BSA (20 mg) in DMF (1 mL) for 4 h and all yields were determined by HPLC.

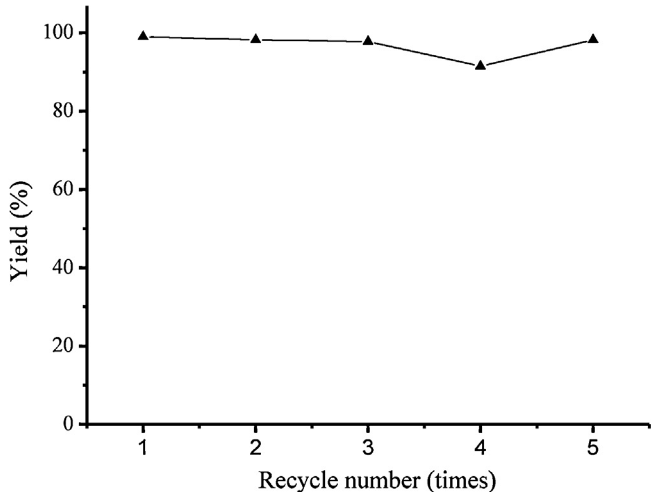

Fig. 4. Reuse of BSA on the model reaction. Reactions were performed with cyclohexanone (1 mmol), malononitrile (1 mmol), sulfur (1 mmol) and BSA (50 mg) in DMF (1 mL) for 4 h and all yields were determined by HPLC.

proceeded smoothly with a wide range of ketones and aldehydes. Comparing different activated nitrile compounds, we can find that the activity of malononitrile is much higher than that of ethyl cyanoacetate (entries 1 and 2; entries 3 and 4; entries 5 and 6; entries 7 and 8; entries 9 and 10, Table 3). Furthermore, the ring size of cyclic ketones may exert an obvious influence. For example, the yield of cyclohexanone, ethyl cyanoacetate and elemental sulfur after 12 h could come up to 77% (entry 2, Table 3). However, when cyclopentanone and cycloheptanone were applied as substrates, the yield dramatically decreased to 21% and 25%, respectively (entries 4 and 6, Table 3). This phenomenon might be ascribed to the higher internal strain of these two cyclic ketones [27]. The chain length of ketone has also shown great influence on the formation of products. In the case of acetone (entry 8, Table 3), compound **3h** was obtained with a moderate yield of 60%, while when butanone was used as ketone under the same condition, a slightly decreased yield was observed (entry 10, Table 3). This difference could be caused by the increasing length of the chain, which might raise the steric hindrance. Phenyl acetaldehyde could also act as a substrate of Gewald reaction. However, its low activity and high hindrance could only lead to relatively low yields.

3.8. Proposed mechanism

According to the previous research, the catalytic activity of BSA is based on the basic character of the amino group present in the side chain of some amino acid residues, especially the lysine residue [28]. Therefore, we investigate the Gewald catalytic activity of several amino acids to gain a further insight of the mechanism of this BSA catalyzed Gewald reaction (entries 1–5, Table 4). Among all the amino acids tested, L-lysine gives the best result with a yield of more than 99% (entry 5, Table 4), which is at the same level of the result of BSA, while other amino acids show much lower activities (entries 1–4, Table 4). According to the previous report of Taylor [29], combining with Brown's sequence data [30], the Gewald reaction is possibly catalyzed by the lysine 200 at the active site of BSA, and a plausible mechanism is proposed (Fig. 5). Generally, the basic active-site features of an apolar pocket and a lysine residue should act as a primitive active site allowing the activities to take place [31]. Firstly, the lysine's amine attacks on the carbonyl group of various substrates leading to the formation of a covalent enamine intermediate **5** [32]. It can also serve as a base to grab the proton

Table 3
Gewald reaction catalyzed by BSA.[a]

Entry	Substrate (**1**)	Substrate (**2**)	Product (**3**)	Reaction time (h)	Yield (%)[b]
1		NC CN		4	>99
2		NC COOEt		12	77[c]
3		NC CN		4	65
4		NC COOEt		12	21[c]
5		NC CN		4	93
6		NC COOEt		12	25[c]
7		NC CN		4	94
8		NC COOEt		12	60[c]
9		NC CN		4	96
10		NC COOEt		12	57[c]
11		NC CN		4	65
12		NC COOEt		12	20[c]

[a] Reactions of ketones or aldehydes **1** (1 mmol), activated nitriles **2** (1 mmol) and elemental sulfur (1 mmol) were performed in DMF (1 mL), under the catalysis of BSA (20 mg) at 50 °C.
[b] Isolated yield.
[c] BSA was applied in 30 mg amount.

of α-methylene nitrile compounds, forming compounds **6** that lack of proton. Then **6** attacks the imino group of **5**. The corresponding α,β-unsaturated nitrile compound is obtained. This step, known as the Knoevenagel condensation, occurs in the presence of acid/base catalyst or a heterogeneous support [33]. Then, the elemental sulfur is activated by lysine's amine and reacts with **7** to obtain **8**. This intermediate is cyclized to form the desired product **4** eventually after a series of process [34].

Fig. 5. Proposed mechanism of the Gewald reaction catalyzed by BSA.

Table 4
Investigation of amino acids.[a]

catalyst, DMF, 50 °C

Entry	Catalyst	Reaction time (h)	Yield (%)[b]
1	L-Glutamic acid	12	Trace
2	L-Threonine	12	18
3	L-Serine	12	20
4	L-Cysteine	12	46
5	L-Lysine	4	>99

[a] Reactions were performed with cyclohexanone (1 mmol), malononitrile (1 mmol), sulfur (1 mmol) and catalysts (20 mg) in DMF (1 mL) at 50 °C.
[b] All yields were determined by HPLC.

4. Conclusion

In conclusion, a simple and efficient method for the synthesis of substituted 2-aminothiophenes via BSA-catalyzed Gewald reaction is described here. A series of functionalized 2-aminothiophenes scaffolds were synthesized in moderate to excellent yields. Owing to the easily accessible and versatile starting material, this method shows great potential in synthesis of many biologically and pharmaceutically active 2-aminothiophenes derivatives.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molcatb.2013.05.014.

References

[1] (a) G.A. Elmegeed, W.W. Wardakhan, M. Younis, N.A. Louca, Arch. Pharm. 337 (2004) 140–147;
(b) K.S.M. Shetty, V. Somashekar, S. Mohan, Asian J. Chem. 16 (2004) 623–627;
(c) K. Harza, J. Saravanan, S. Mohan, Asian J. Chem. 19 (2007) 3541–3544;
(d) D. Singh, S. Mohan, P.C. Sharma, J. Saravanan, Acta Pharm. Sci. 49 (2007) 29–38.

[2] R.W. Sabnis, D.W. Rangnekar, N.D. Sonawane, J. Heterocycl. Chem. 36 (1999) 333–345.

[3] R.M. Angell, F.L. Atkinson, M.J. Brown, T.T. Chuang, J.A. Christopher, M. Cichy-Knight, A.K. Dunn, K.E. Hightower, S. Malkakorpi, J.R. Musgrave, M. Neu, P. Rowland, R.L. Shea, J.L. Smith, D.O. Somers, S.A. Thomas, G. Thompson, R.L. Wang, Bioorg. Med. Chem. Lett. 17 (2007) 1296–1301.

[4] (a) S.M. Cohen, J.L. Duffy, C. Miller, B.A. Kirk, M.R. Candelore, V.D.H. Ding, G. Kaczorowski, L.M. Tota, J.G. Werrmann, M. Wright, E.R. Parmee, J.R. Tata, B.B. Zhang, Bioorg. Med. Chem. 14 (2006) 1506–1517;
(b) R. Romagnoli, P.G. Baraldi, M.D. Carrion, C.L. Cara, D. Preti, F. Fruttarolo, M.G. Pavani, M.A. Tabrizi, M. Tolomeo, S. Grimaudo, A. Di Cristina, J. Balzarini, J.A. Hadfield, A. Brancale, E. Hamel, J. Med. Chem. 50 (2007) 2273–2277.

[5] K. Gewald, E. Schinke, H. Bottcher, Chem. Ber. Recl. 99 (1966) 94–100.

[6] W.L. Silva, M.d.C.A. de Lima, S.L. Galdino, I.R. Pitta, C.A. De Simone, Acta Crystallogr. E: Struct. Rep. Online 67 (2011) O3161–U3836.

[7] A.A. Abu-Hashem, K.M. Abu-Zied, M.F. El-Shehry, Monatsh. Chem. 142 (2011) 539–545.

[8] T. Wang, X.G. Huang, J. Liu, B. Li, J.J. Wu, K.X. Chen, W.L. Zhu, X.Y. Xu, B.B. Zeng, Synlett (2010) 1351–1354.

[9] M. Fujita, T. Seki, N. Ikeda, Bioorg. Med. Chem. Lett. 12 (2002) 1897–1990.

[10] M. Feroci, I. Chiarotto, L. Rossi, A. Inesi, Adv. Synth. Catal. 350 (2008) 2740–2746.

[11] G.M. Castanedo, D.P. Sutherlin, Tetrahedron Lett. 42 (2001) 7181–7184.

[12] (a) M. Xu, F. Zhang, B.K. Liu, Q. Wu, X.F. Lin, Chem. Commun. 20 (2007) 2078–2080;
(b) J.L. Wang, X. Li, H.Y. Xie, B.K. Liu, X.F. Lin, J. Biotechnol. 145 (2010) 240–243;
(c) Q. Wu, J.M. Xu, X. Li, J.L. Wang, X.F. Lin, Adv. Synth. Catal. 351 (2009) 1833–1841;
(d) A. Talukdar, Y.J. Zhao, W. Lv, A. Bacher, B. Illarionov, M. Fischer, M. Cushman, J. Org. Chem. 77 (2012) 6239–6261.

[13] M. De Rosa, S. Di Marino, A.M. D'Ursi, M. Strianese, A. Soriente, Tetrahedron 68 (2012) 3086–3091.

[14] M. Bocola, F. Schulz, F. Leca, A. Vogel, M.W. Fraaije, M.T. Reetz, Adv. Synth. Catal. 347 (2005) 979–986.

[15] E. Busto, V. Gotor-Fernandez, V. Gotor, Org. Process Res. Dev. 15 (2011) 236–240.

[16] N. Gaggero, D.C.M. Albanese, G. Celentano, S. Banfi, A. Aresi, Tetrahedron: Asymmetry 22 (2011) 1231–1233.

[17] M.A. Zolfigol, H. Vahedi, A. Massoudi, S. Sajjadifar, O. Louie, N. Javaherneshan, Clin. Biochem. 44 (2011) S219.

[18] (a) N. Sharma, U.K. Sharma, R. Kumar, N. Katoch, R. Kumar, A.K. Sinha, Adv. Synth. Catal. 353 (2011) 871–878;
(b) U.K. Sharma, N. Sharma, R. Kumar, A.K. Sinha, Amino Acids 44 (2013) 1031–1037.

[19] E.S. Fondjo, D. Dopp, G. Henkel, Tetrahedron 62 (2006) 7121–7131.

[20] M. Sridhar, R.M. Rao, N.H.K. Baba, R.M. Kumbhare, Tetrahedron Lett. 48 (2007) 3171–3172.

[21] K.S. Kumar, S. Chamakuri, P. Vishweshwar, J. Iqbal, M. Pal, Tetrahedron Lett. 51 (2010) 3269–3273.

[22] F. Pak, D. Ekinci, F. Tümer, Ü. Demir, Macromol. Chem. Phys. 208 (2007) 2367–2374.

[23] M. Gutschow, L. Kuerschner, U. Neumann, M. Pietsch, R. Loeser, N. Koglin, K. Eger, J. Med. Chem. 42 (1999) 5437–5447.

[24] K. Gewald, E. Schinke, H. Botcher, Chem. Ber. 99 (1966) 94–100.

[25] K. Gewald, Chem. Ber. 98 (1965) 3571–3577.

[26] S. Hesse, G. Revelant, S. Dunand, G. Kirsch, Synthesis 18 (2011) 2935–2940.

[27] (a) H.C. Brown, M. Gerstein, J. Am. Chem. Soc. 72 (1950) 2926–2933;
(b) H.C. Brown, M.J. Borkowski, J. Am. Chem. Soc. 74 (1952) 1894–1902;
(c) H.C. Brown, J.H. Brewster, H. Shechter, J. Am. Chem. Soc. 76 (1954) 467–474.

[28] T. Peters Jr., All about Albumin: Biochemistry, Genetics, and Medical Applications, Academic Press, New York, NY, 1996.
[29] (a) R.P. Taylor, J.B. Vatz, J. Am. Chem. Soc. 95 (1973) 5819–5820;
(b) R.P. Taylor, V. Chau, C. Bryner, S. Berga, J. Am. Chem. Soc. 97 (1975) 1934–1943.
[30] (a) J.R. Brown, Fed. Proc. Abst. (1975) 2105;
(b) P.Q. Behrens, A.M. Spierkerman, J.R. Brown, Fed. Proc. Abst. (1975) 2106.
[31] (a) F. Hollfelder, A.J. Kirby, D.S. Tawfik, Nature 383 (1996) 60–62;
(b) R.P. Taylor, J. Am. Chem. Soc. 98 (1976) 2684–2686.
[32] L.C. James, D.S. Tawfik, Protein Sci. 10 (2001) 2600–2607.
[33] (a) R. Gupta, M. Gupta, S. Paul, R. Gupta, Bull. Korean Chem. Soc. 30 (2009) 2419–2421;
(b) X. Xin, X. Guo, H. Duan, Y. Lin, H. Sun, Catal. Commun. 8 (2007) 115–117;
(c) J.R. Harjani, S.J. Nara, M.M. Salunkhe, Tetrahedron Lett. 43 (2002) 1127–1130;
(d) Y. Zhang, Y. Zhao, C. Xia, J. Mol. Catal. A: Chem. 306 (2009) 107–112;
(e) G. Postole, B. Chowdhury, B. Karmakar, K. Pinki, J. Banerji, A. Auroux, J. Catal. 269 (2010) 110–121.
[34] (a) H.P. Buchstaller, C.D. Siebert, R.H. Lyssy, I. Frank, A. Duran, R. Gottschlich, C.R. Noe, Monatsh. Chem. 132 (2001) 279–293;
(b) N.P. Peet, S. Sunder, R.J. Barbuch, A.P. Vinogradoff, J. Heterocycl. Chem. 23 (1986) 129–134.