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Efficient synthesis of unsymmetrical trisubstituted 1,3,5-triazines catalyzed by hemoglobin

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ABSTRACT

Background: 1,3,5-triazines are important bioactive compounds that have been extensively studied in organic chemistry. In this work, a green and efficient process for the synthesis of unsymmetrical trisubstituted 1,3,5-triazines from isothiocyanate (**1**) with amidines (**2**) and 1,1,3,3-tetramethylguanidine (TMG, 3) was developed.
Results: Under optimal conditions (isothiocyanate (0.1 mmol), amidines (0.1 mmol), 1,1,3,3-tetramethylguanidine (0.1 mmol), DMSO (1 mL), hemeprotein (heme concentration: 0.05 mol%), TBHP (3 equiv), room temperature, 10 min), high yields of 1,3,5-triazines (81%–96%) could be obtained when HbRb (Hemoglobin from rabbit blood) was used as the catalyst.
Conclusion: This enzymatic method demonstrates the great potential for the synthesis of unsymmetrical trisubstituted 1,3,5-triazines and extends the application of enzyme catalytic promiscuity in organic synthesis.

Introduction

The core skeleton of 1,3,5-triazines is a very important scaffold in molecules exhibiting a broad spectrum of biological and pharmacological activities, such as anticancer, antiviral, antifungal, antimalarial, anti-inflammatory, and anti-angiogenesis activities [1–7]. 1,3,5-Triazines and their substitutions are crucial to the generation and escalation of activity. Accordingly, the synthesis of unsymmetrical 1,3,5-triazines with different bioactive substitutes is significant for research on organic and pharmaceutical chemistry.

Traditionally, cyanuric chloride is used to synthesize unsymmetrical trisubstituted triazines, but stepwise replacement of the three chlorines often produces mixtures of compounds, leading to serious separation and purification problems [8,9]. Various alternative methods can reportedly overcome these problems. For example, the acylation/cyclization of biguanides with appropriate esters and the transition-metal-catalyzed reactions of biguanides and selected alcohols or dihaloalkenes can enable successful syntheses of trisubstituted unsymmetrical triazines (Scheme 1, A1 and A2) [10,11]. The copper-catalyzed cyclization of amidines with a C1 source (N, N-dimethylformamide or trialkylamines) has been performed to synthesize trisubstituted unsymmetrical triazines in moderate yields (Scheme 1, B1 and B2) [12,13]. Xu and co-workers designed a base-mediated three--component reaction of imidates, guanidines, and amides/aldehydes to synthesize unsymmetrical 1,3,5-triazin-2-amines under thermal conditions (Scheme 1, C1) [14]. Vasu et al. presented the one-pot synthesis of trisubstituted 1,3,5-triazines starting from isothiocyanates, N, N-diethylamidines, and carbamidines in the presence of mercury (II) chloride to generate desired 1,3,5-triazines in good to moderate yields (Scheme 1, D1) [15]. Recently, Liang's group developed a highly efficient visible-light-promoted [5 + 1] annulation of biguanides and perfluoroalkyl iodides to construct perfluoroalkyl-s-triazines under mild reaction conditions (Scheme 1, A3) [16]. Another visible-light-promoted [3 + 1+2] annulation of isothiocyanates, amidines, and guanidines has been explored by Zhang's group for the synthesis of unsymmetrical trisubstituted 1,3,5-triazines in moderate yields (Scheme 1, D2) [17]. However, all these methods suffer from drawbacks such as harsh reaction conditions, toxic metal catalysts, complicated products, difficult separation, low yields, and long reaction time. Considering these disadvantages, developing environmentally friendly and efficient methods of preparing unsymmetrical trisubstituted 1,3,5-triazines remains fascinating for researchers of medicinal and synthetic chemistry.

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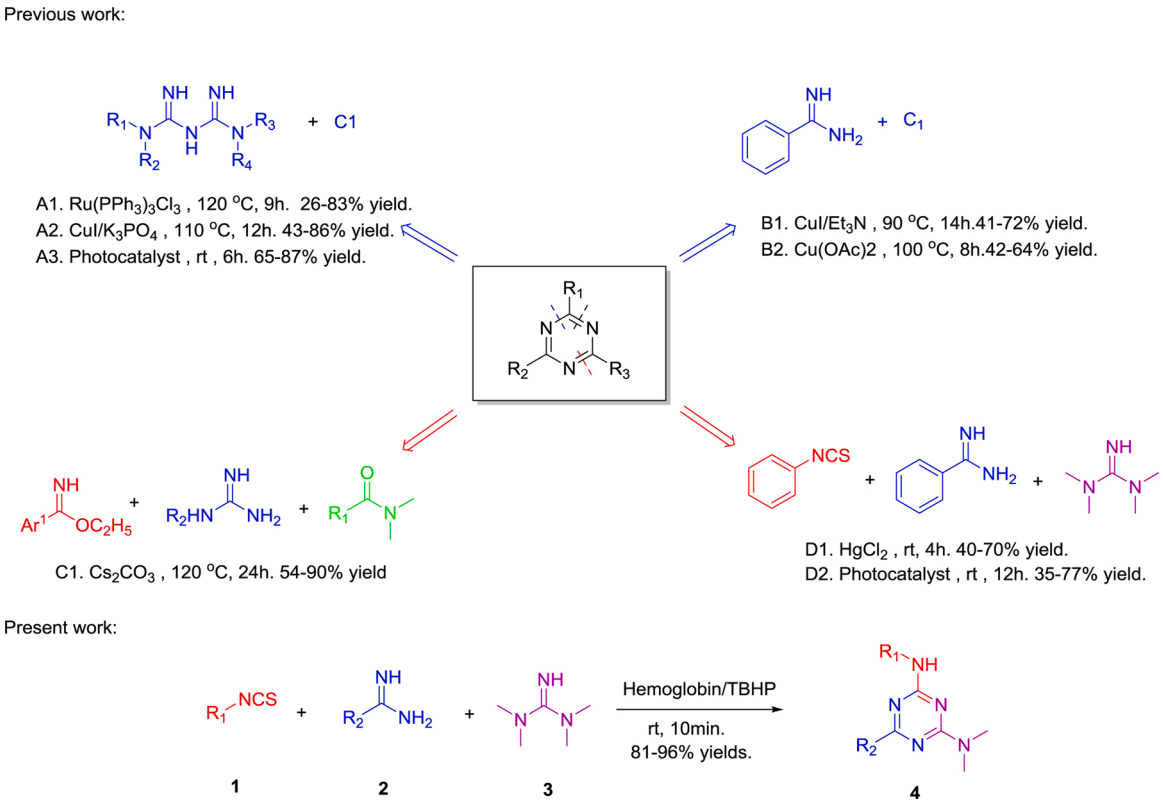


Scheme 1. Synthesis of unsymmetrical trisubstituted 1,3,5-triazines catalyzed by hemoglobin.

Biocatalysis is extensively used to synthesize diverse fine chemicals as an efficient and green method with high efficiency, mild reaction conditions, and environmental friendliness [18]. However, the main reason limiting the application of biocatalysis is the breadth of reactions catalyzed by proteins. Thus, discovering new non-natural reactions is gaining increased research attention [19–21]. In this area, hemoproteins demonstrate huge potential to catalyze non-natural reactions, and many related studies have been conducted. Such reactions include oxidative (hydroxylation, epoxidation, oxidative cyclization, and sulfoxidation) [22–25] and carbene-mediated reactions [26–29]. New catalytic abilities can further be enhanced by extensive protein engineering [30,31]. Hemoglobin, a hemoprotein responsible for transporting oxygen in the blood of vertebrates, is low cost, stable, and commercially available, rendering advantageous is use as an ideal biocatalyst in organic synthesis. In our previous reports, hemoglobin has been utilized to synthesize various nitrogen heterocycles, such as benzoxazoles, indolizines, and quinoxalines [32–34]. As part of our studies towards the development of hemoglobin-catalyzed non-natural reactions, we report herein a hemoglobin-catalyzed multicomponent reaction to synthesize unsymmetrical trisubstituted 1,3,5-triazines (Scheme 1). To the best of our knowledge, this report is the first one on a biocatalysis method of synthesising 1,3,5-triazines. Our hemoglobin-catalyzed protocol can produce higher yield (81 %–96 %) and shorter reaction time (10 min) than other methods.

Experimental

Chemicals

Horseradish peroxidase, Myoglobin from equine heart, Hemoglobin from porcine blood, Hemoglobin from bovine blood, Cytochrome C from horse heart muscle, Cytochrome C from bovine heart muscle, Cytochrome C from swine heart muscle was purchased from Shanghai Yuan Ye Biological Technology Company. TMG, isothiocyanate, amidines were purchased from Bide Pharmatech Ltd. (Shanghai, China). All the other chemical reagents were purchased from Shanghai Chemical Reagent Company (Shanghai, China). All the commercially available reagents and solvents were used without further purification. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a 400 MHz spectrometer in $CDCl_3$, and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on 100 MHz spectrometer in $CDCl_3$. Chemical shifts for protons are reported in parts per million downfield from tetramethylsilane (TMS) and are referenced to residual protium in the NMR solvent ($CHCl_3$ = δ 7.26 ppm). Chemical shifts for carbon are reported in parts per million downfield from tetramethylsilane (TMS) and are referenced to the carbon resonances of the solvent residual peak ($CDCl_3$ = δ 77.16 ppm). NMR data are presented as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant in Hertz (Hz), integration. The experiments were performed triplicate, and all data

Table 1
Hemoprotein-catalyzed synthesis of 4a through a multicomponent reaction.[a]

Entry	Catalyst	Isolated yield (%)
1	HRP	71
2	MYO	46
3	HbSw	63
4	HbBv	81
5	HbHm	74
6	HbRb	91
7	Cyt. C Hs	92
8	Cyt. C Bv	81
9	Cyt. C Sw	73
10	Apo-HbRb	3
11	Denatured HbRb [b]	7
12	–	4
13	Fe (TPP)Cl [c]	28 [d]
14	Hemin [c]	32 [d]
15	$FeCl_3$ [c]	21 [d]
16	Fe $(OTf)_3$ [c]	19 [d]

Abbreviation: HRP (Horseradish peroxidase); MYO (Myoglobin from equine heart); HbSw (Hemoglobin from swine blood); HbBv (Hemoglobin from bovine blood); HbHm (Hemoglobin from human); HbRb (Hemoglobin from rabbit blood); Cyt.C Hs (Cytochrome C from horse heart); Cyt.C Bv (Cytochrome C from bovine heart); Cyt.C Sw (Cytochrome C from swine heart muscle).

[a] **1a** (0.1 mmol), **2a** (0.1 mmol), **3** (1,1,3,3-tetramethylguanidine; 0.1 mmol.), hemoprotein (heme concentration: 0.05 mol%), TBHP (3 equiv.), dimethylsulfoxide (1 mL), room temperature, 10 min.

[b] Heating HbRb in boiling water for 5 h.

[c] Catalysts loading: 0.5 mol%.

[d] Reaction time prolong for 1 h.

were obtained based on the average values. Mass spectra were recorded on the Bruker MicrOTOF Q II and an Orbitrap Fusion™ Tribrid™ mass spectrometer (Thermo Scientific, San Jose, CA, U.S.A.) coupled with HESI ion source.

Preparation of apo Hb

To the ice-cold, salt-free Hb solution containing sufficient 0.1 N HCl to give pH 2, is added an equal volume of ice-cold methyl ethyl ketone, and the mixture is shaken for a short time. On standing in the cold, separation takes place into a ketonic supernatant containing all the heme, and the aqueous layer containing all the protein, which is dialysed against water to remove the dissolved ketone.

General procedure for the synthesis of 1,3,5-triazines

To a mixture of isothiocyanates (0.1 mmol), 1,1,3,3-tetramethylguanidine (1.0 equiv), in DMSO (1 mL), hemoproteins (heme concentration: 0.05 mol%), benzimidamide hydrochloride (1.0 equiv), was added. Then 3 equiv. of TBHP was added dropwise into the above mixture and 10 min stirring was allowed at room temperature. The reaction was monitored by TLC. When the reaction was complete, the crude mixture was added water and extracted with ethyl acetate. Then the organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Finally, the desired product was obtained by flash column chromatography with petroleum ether/ethyl acetate (4/1) as an eluent. All the isolated products were well characterized by their ^1H, ^{13}C NMR and MS spectral analysis.

Table 2
Effect of solvents on the hemoglobin-catalyzed synthesis of **4a**.[a]

Entry	Solvent	Isolated yield (%)
1	n-Hexane	26
2	Toluene	33
3	Ethyl acetate	31
4	Dichloromethane	85
5	Acetone	87
6	Ethanol	72
7	Acetonitrile	65
8	N, N-Dimethylformamide	82
9	Dimethylsulfoxide	91
10	Water	47

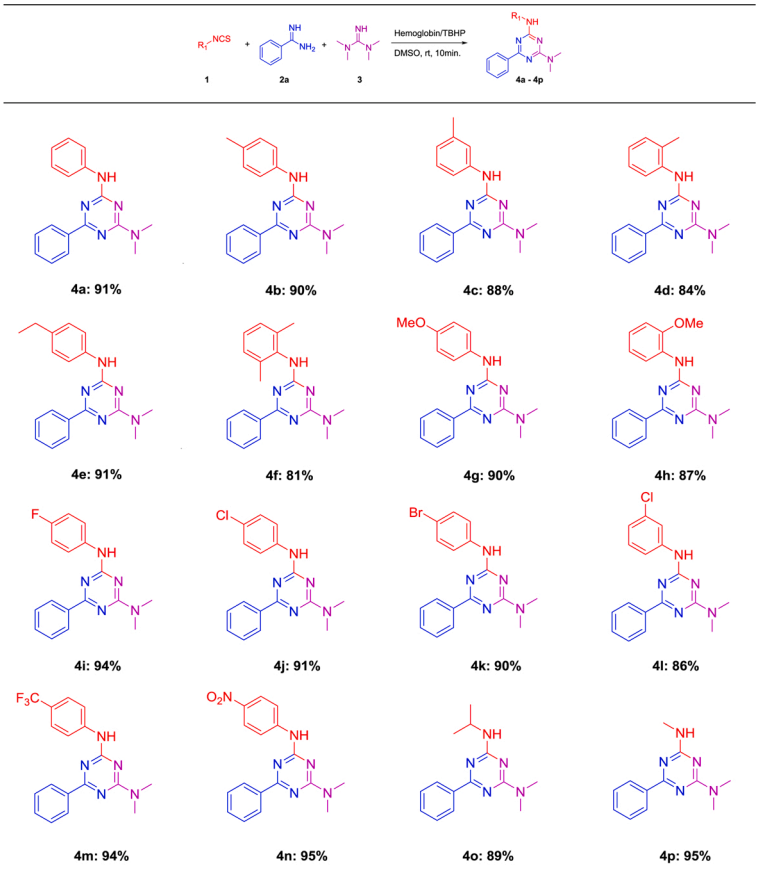
[a] Reaction conditions: **1a** (0.1 mmol), **2a** (0.1 mmol), **3** (1,1,3,3-tetramethylguanidine) (0.1 mmol), HbRb (heme concentration: 0.05 mol%), TBHP (3 equiv.), Solvent (1 mL), room temperature, 10 min.

Results and discussion

Initially, we selected the reaction of isothiocyanatobenzene (**1a**) with benzimidamide (**2a**) and 1,1,3,3-tetramethylguanidine (TMG, **3**) as the model to optimise the reaction conditions with tert-butyl hydroperoxide (TBHP) as an oxidant. As shown in Table 1, all hemoproteins demonstrated good catalytic performance at room temperature, and the catalytic activities primarily depended on the type and origin of hemoproteins. Among the hemoproteins used, hemoglobin from bovine blood (HbRb) and cytochrome C from horse heart muscle afforded the highest yield (91 %–92 %) of triazine with only 0.05 mol% (heme concentration) catalyst loading (entries 6–7). Considering the protein cost, we adopted HbRb as the optimum biocatalyst for further study. When Apo-HbRb or denatured HbRb was used as the catalyst (entries 10–11), the yield was similar to that of the blank experiment (entry 12), suggesting that the special active conformation and heme center of hemoglobin were critical in this reaction. Subsequently, we evaluated the utility of several commercially available ferric porphyrins (Fe (TPP)Cl and Hemin) and ferric salt catalysts ($FeCl_3$ and Fe $(OTf)_3$). Product **4a** was also detected but in relatively lower yields even with prolonged reaction time to 1 h (entries 13–16), it was demonstrated different from Fenton chemistry and there is a more active free radical (Compound I). Compared with the above chemical catalysts, HbRb exhibited much higher catalytic efficiency towards this multicomponent reaction. The loading of HbRb and oxidant ratio were then investigated, and 0.05 mol % HbRb and 3 equiv. TBHP were found to be ideal (Table S1). We also investigated the effect of reaction time on products yield, and the results are shown in Fig. S1. The yield of **4a** increased with increasing reaction time (0–10 min). Nevertheless, longer reaction time did not significantly increased the yield. Thus, the reactions were conducted with 10 min as the optimal reaction time for the remainder of this method.

Reaction medium is also an important aspect in a protein-catalyzed reaction because it influences the distribution of all reactants and intermediates and the activity of proteins by varying the protein conformation [35], and the hemoprotein have good stability and long-term storage in organic solvent [36]. In this study, the effect of reaction medium on the hemoglobin-catalyzed reaction was investigated, and the results are presented in Table 2. The yield of 4a was dramatically affected by reaction medium, and dimethylsulfoxide (DMSO) was the most suitable solvent for the reaction and DMSO is an environmentally preferable solvent which off ;ers advantages in terms of health and

Table 3
Scope of isothiocyanate compounds.[a]



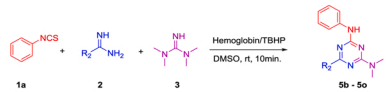
[a] Reaction conditions: **1** (0.1 mmol), **2a** (0.1 mmol), **3** (0.1 mmol), HbRb (heme concentration: 0.05 mol%), TBHP (3 equiv.), DMSO (1 mL), room temperature, 10 min.

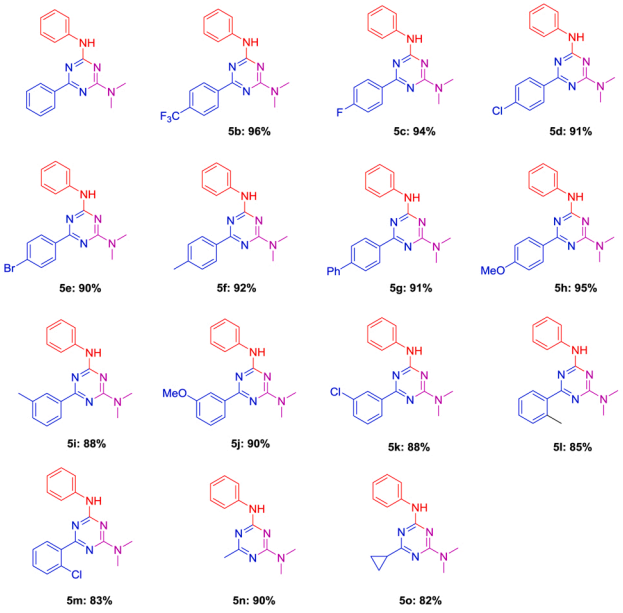
safety [37]. The stability of HbRb in DMSO were then investigated. The results indicated the good stability of HbRb in DMSO and confirmed an active role of the protein in the reaction instead of free hemin released from the protein (Figs. S2-S3). Furthermore, we scaled up the hemoglobin-catalyzed reaction by 500-fold under the optimum conditions [DMSO (500 mL), isothiocyanatobenzene (1a, 50 mmol), benzimidamide (2a, 50 mmol), 1,1,3,3-tetramethylguanidine (3, 50 mmol), HbBv (heme concentration of 0.1 mol %), and TBHP (3 equiv.) at room temperature]. The yield of the corresponding triazine 4a reached 95 % in a short reaction time (10 min). This result indicated the high potential of the efficient and green method for practical application.

Under the optimum reaction conditions, the scope and generality of the reaction with respect to the isothiocyanates **1**, amidines **2,** and TMG **3** were investigated. Firstly, a range of substituted isothiocyanatobenzenes **1** were examined in the presence of **2a** and **3**. As shown in Table 3, isothiocyanatobenzenes with various substituents on the aromatic ring were well tolerated, affording the desired triazines (**4b**–**4n**) in high yields (81 %–94 %). The electronic effects of the substituents had a certain influence on the reaction, and isothiocyanatobenzenes with an electron-withdrawing group on the benzene ring afforded higher yields than those with an electron-donating group on benzene. Steric effects were also found to affect the yield of the triazines as demonstrated by the *ortho*-substituted isothiocyanates, producing a diminished yield of the products compared with their *meta*- and *para*-substituted counterparts. Furthermore, aliphatic isothiocyanates (isothiocyanatomethane and 2-isothiocyanatopropane) were successfully utilized for this reaction to produce the corresponding products in excellent yields (**4o**–**4p**).

To further demonstrate the applicability of the method, the scope of amidines **2** in this hemoglobin-catalyzed reaction was also explored. All substituted benzamidines were reacted smoothly with isothiocyanatobenzene **1a** and TMG **3** smoothly to afford the corresponding triazines in high yields. As shown in Table 4, the substituents had weak electronic effects and strong steric effects on the reaction. The *para*-substituted(**5b**-**5 h**) substances afforded the products in higher yields than the *ortho*- (**5i**-**5k**) or *meta*-substituted(5 L-**5 m**) benzamidines. Meanwhile, aliphatic amidines (acetimidamide and cyclopropanecarboximidamide) were subjected to this reaction system and

Table 4
Scope of amidines.[a]





[a] Reaction conditions: **1a** (0.1 mmol), **2** (0.1 mmol), **3** (0.1 mmol), HbRb (heme concentration: 0.05 mol%), TBHP (3 equiv.), DMSO (1 mL), room temperature, 10 min.

provided **5n** and **5o** in high yields.

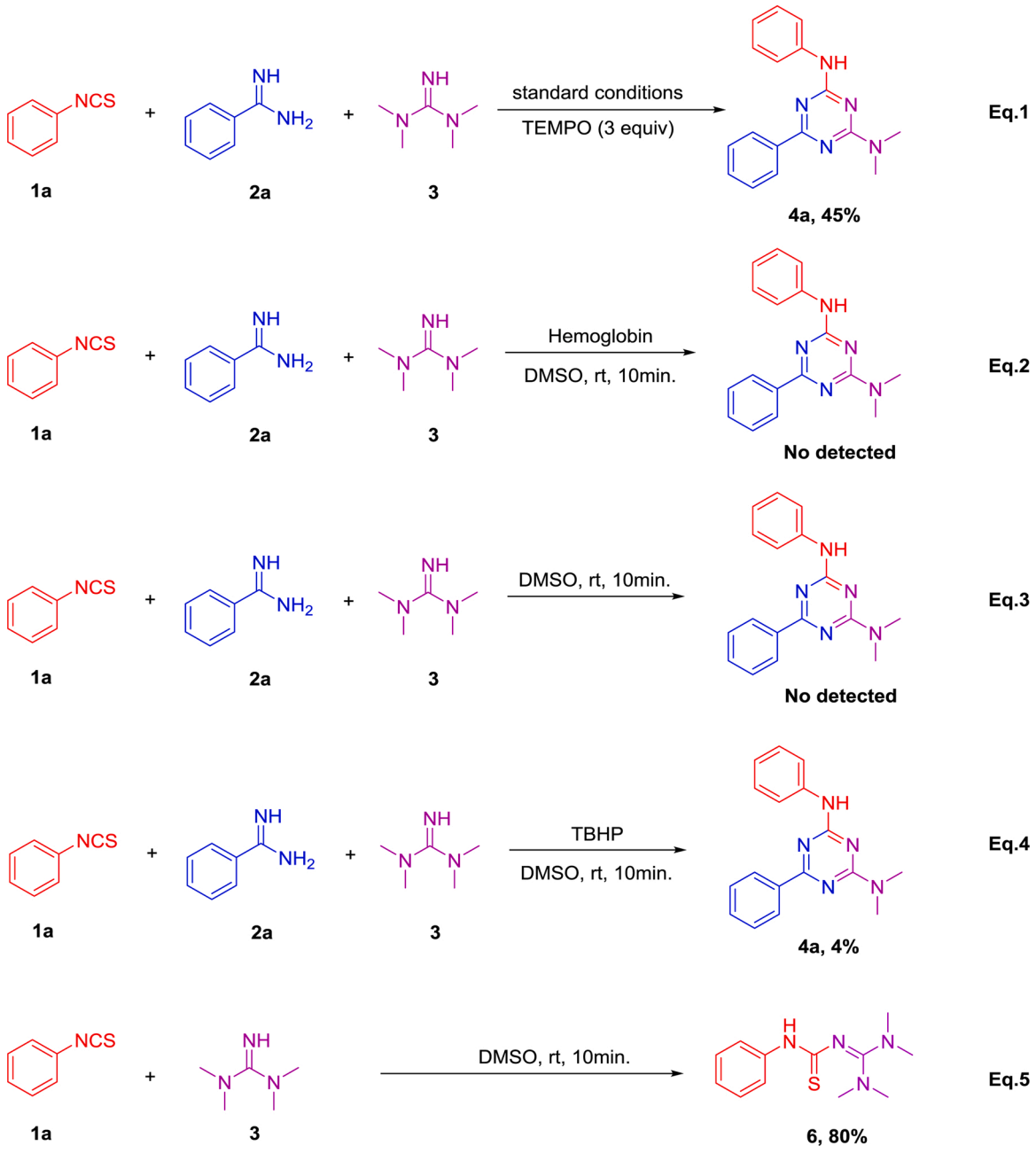
To probe the mechanism of this reaction, some control experiments were performed, and the results are shown in Scheme 2. Firstly, addition of the radical scavenger TEMPO (3 equiv.) dramatically decreased the yield of **4a** (Eq. 1). This result implied the involvement of a radical pathway in this hemoglobin-catalyzed reaction. No corresponding triazine **4a** was obtained when TBHP was absent in this system (Eqs. 2 and 3), and only 4% yield of **4a** was observed (Eq. 4) in the presence of TBHP without hemoglobin. These results indicated that hemoglobin obviously accelerated the reaction and TBHP was necessary to maintain the catalytic ability of hemoglobin. Moreover, the reaction of isothiocyanatobenzene **1a** with TMG **3** afforded compound **6** in 80 % yield without hemoglobin and oxidant (Eq. 5), and it was also detected during this hemoglobin-catalyzed reaction. Therefore, compound **6** was a key intermediate in this reaction pathway.

Based on literature and our experimental results [17,38–41], a possible mechanism of the hemoglobin-catalyzed synthesis of trisubstituted 1,3,5-triazines was proposed (Scheme 3). Initially, the heme of hemoglobin is converted to compound I in the presence of TBHP. This potent oxidant intermediate facilitates hydrogen atom transfer (HAT) from the substrate **2a** to the cofactor, generating the Fe (IV)-hydroxide species (compound II) and the radical intermediates **2a′**. Then, the reaction of isothiocyanatobenzene **1a** and TMG **3** occurrs spontaneously and quickly affords intermediate **6**, which is oxdized by compound II to the produce corresponding radical intermediates **6′**. Radical intermediate **2a′** further reacts with radial intermediate **6′** to generate a key intermediate **7** through the radical coupling process. This intermediate is then further converted to the product **4a** by the elimination of H_2S and intramolecular cyclization.

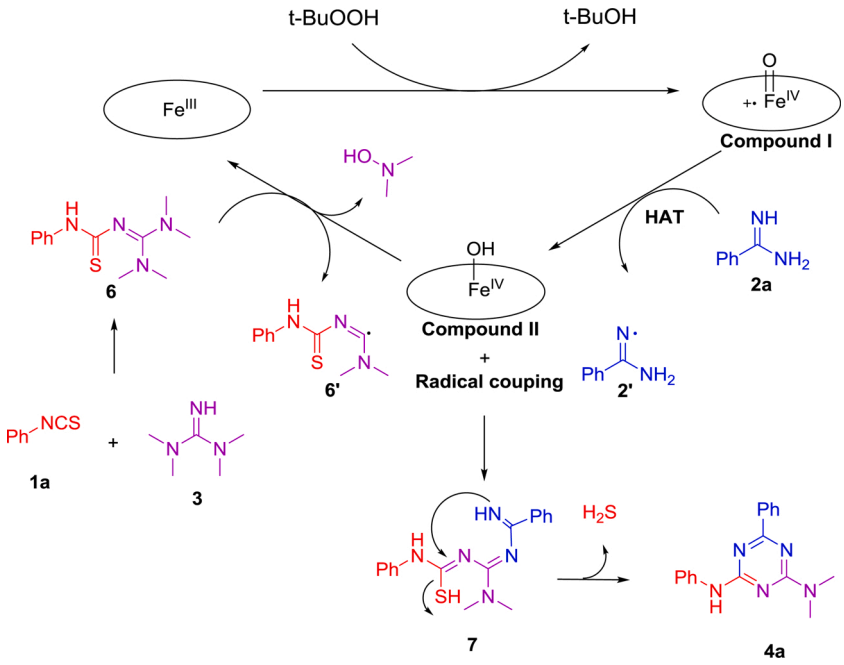
In conclusion, this work demonstrated an efficient and environmentally friendly strategy for the construction of unsymmetrical trisubstituted 1,3,5-triazines at room temperature. This work presented a new hemoglobin capable of catalyzing the synthesis of 1,3,5-triazines with higher yield (81 %–96 %) and shorter reaction time (10 min) than ever reported, thereby demonstrating the huge potential of hemoglobin as an optimum source of novel biocatalysts for new non-natural reaction.

CRediT authorship contribution statement

Fengxi Li: Conceptualization, Methodology, Formal analysis, Software. **Chunyu Wang:** Software. **Yaning Xu:** Investigation, Resources. **Zixian Zhao:** Visualization. **Jiali Su:** Writing - original draft. **Chenhan Luo:** Writing - review & editing. **Yujie Ning:** Data curation. **Zhengqiang Li:** Software. **Chen Li:** Supervision. **Lei Wang:** Supervision.



Scheme 2. Control experiments.



Scheme 3. Plausible mechanism of hemoglobin - catalyzed reaction.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mcat.2021.111519.

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