Optimization of 2-amino-4-methyl-5-ethoxycarbonyl Thiazole Synthesis

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Abstract

The global rise of antibiotic resistance poses a major public health threat, highlighting the need for new antibacterial agents. Thiazoles have attracted attention because of their antibacterial, antifungal, and pharmaceutical potential. However, traditional thiazole synthesis methods, such as the Hantzsch synthesis (1887), typically yield only (18-20)%, while optimization strategies remain limited. Preliminary trials under conventional conditions yielded only 22.80%. This study aimed to optimize the synthesis of 2-amino-4-methyl-5-ethoxycarbonyl thiazole using Response Surface Methodology (RSM). Three factors – thiourea-to-ethyl acetoacetate molar ratio, reaction temperature, and reaction time – were investigated using Central Composite Design (CCD). The product was purified by solvent crystallization and characterized by UV-Vis, IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy. Under optimized conditions (molar ratio 1.23, 77 °C, 5.75 h), the yield reached (50.84 ± 1.65)%. These results demonstrate the effectiveness of RSM in improving thiazole synthesis.

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Keywords

Thiazole synthesis; optimization; response surface methodology.

1 Introduction

In the context of growing global antimicrobial resistance (AMR) in Viet Nam, developing novel compounds with potent activity is an urgent priority to protect public health [1]. Thiazole derivatives present a broad spectrum of biological activities, including antibacterial, antifungal, and antioxidant [2]. However, conventional routes such as the Hantzsch, Garbiel, and Cook-Heilbron reactions suffer from poor parameter control, low yields (approximately (18-20)%), and high resource consumption [3]. Therefore, systematically optimizing synthetic parameters is therefore essential to boost productivity, ensure structural purity, and support sustainable pharmaceutical manufacturing [3]. Response Surface Methodology (RSM) offers a powerful approach to model reaction kinetics and optimize operational conditions with minimized experimental trials, yet its application to thiazole synthesis has remained underexplored domestically [4]. To align with Viet Nam's pharmaceutical development goals, this study employed a Central Composite Design (CCD) framework to model and optimize reaction conditions for 2-amino-4-methyl-5-ethoxycarbonyl thiazole, a key intermediate for bioactive thiazole derivatives.

2 Research Methodology

2.1 Investigation of the Synthetic Process

The seminal work published by Hantzsch and Weber (1887) described the occurrence of the thiazole synthesis reaction, but did not specify essential parameters, such as temperature, solvent, or reaction

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time [5]. Subsequent standardization of the reaction conditions was reported by Katritzky and Rees (1996), who indicated that the reaction is typically conducted by refluxing α-haloketone and thioamide at approximately 70 °C for 3 h [6]. These conditions were selected as representative average values within the reported temperature and time ranges to ensure an optimal balance between reaction yield and practical applicability in synthetic processes.

Accordingly, this study investigates the synthesis of 2-amino-4-methyl-5-ethoxycarbonyl thiazole from thiourea, ethyl acetoacetate (EEA), N-bromosuccinimide, and benzoyl peroxide under the aforementioned conditions (Figure 1)

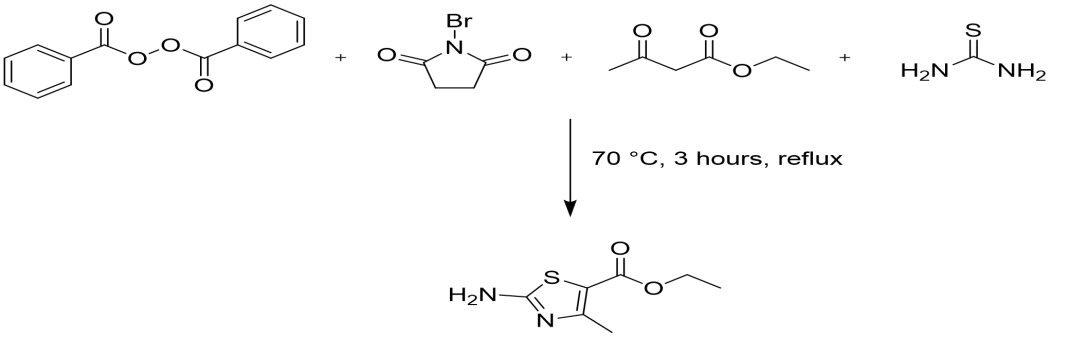


Figure 1 Synthetic pathway for 2-amino-4-methyl-5-ethoxycarbonyl thiazole

- Synthetic Procedure [7]:

In a 100 mL round-bottom flask, 1.7798 g (0.01 mol) of N-bromosuccinimide and 2.4223 g (0.01 mol) of benzoyl peroxide were dissolved in 5 mL of ethyl acetoacetate under reflux. 0.7612 g (0.01 mol) of thiourea was added dropwise, and refluxing was continued at 70 °C for 3 h. The resulting precipitate was collected by filtration, dissolved in distilled water containing 10% HCl, neutralized with saturated $NaHCO_3$ solution, purified via solvent crystallization, and dried at 50 °C.

- Preliminary Purity Assessment by Thin-Layer Chromatography (TLC)

Stationary phase: Pre-coated Silica gel 60 F_{254} plates.

Mobile phase: Developing solvent systems.

2.2 Experimental Design Using RSM - CCD

Preliminary parameters were identified based on CN102079732B (2011), guided by the conventional Hantzsch-type synthesis conditions described by Katritzky and Rees (1996), in which the reaction is typically conducted at approximately 70 °C for about 3 h using equimolar amounts of reactants. Building on these reference conditions, three independent variables were systematically expanded and evaluated across five levels ($-\alpha$, $+\alpha$, 0, +1, −1) for response surface optimization: the molar ratio of reactants X1 from 1.0 to 1.5, reaction temperature X2 (°C) from 70 to 90 °C, and reaction time X3 (h) from 3 h to 9 h. The yield of purified and dried 2-amino-4-methyl-5-ethoxycarbonyl thiazole Y1 was defined as the dependent variable [8]. Ethyl acetoacetate served as both reactant and solvent, with its molar ratio calculated based on maximum reacting moles (0.01 mol).

The total number of experiments was determined using the formula $N = 2k + 2k + c$ ($N = 20$, $k = 3$, $c = 6$), where k is the number of investigated factors, 2k is the number of additional star-point experiments, and c is the number of replicated runs at the center point (0). The distance from the center to the star point $\alpha = \alpha k14$ ($k = 3$, $\alpha = 1.68$), depends on the number of factors selected. This design ensures rotatability, maintaining a uniform prediction variance at equal distances from the design center, irrespective of data orientation [9].

Accordingly, a total of 20 experiments were conducted. This included 8 runs at the cube vertices, 6 runs replicated at the center to evaluate experimental error, and 6 additional runs at the star points positioned at a distance α from the center (Table 1).

The resulting multivariate regression equation models incorporates factor interactions to predict maximum yield under optimal processing conditions.

Table 1 Coded Matrix of Factor Levels Used in the Optimization Study

Symbol	Source	Value				
		Axial points (−α)	Factorial points (−1)	Center points (0)	Factorial points (+1)	Axial points (+α)
A	Mole ratio of Thiourea : EAA (X1)	0.83	1	1.25	1.5	1.67
B	Temperature (°C) (X2)	63	70	80	90	97
C	Time (h) (X3)	0.96	3	6	9	11.04

2.3 Synthesis of 2-amino-4-methyl-5-ethoxycarbonyl Thiazole according to the CCD

In a 100 mL round-bottom flask, 1.7798 g (0.01 mol) of N-bromosuccinimide and 2.4223 g (0.01 mol) of benzoyl peroxide were first added, then 5 mL of ethyl acetoacetate was added. A reflux condenser was assembled, and the mixture was stirred without heating. Thiourea was then slowly added to the reaction mixture at a molar ratio of thiourea to ethyl acetoacetate corresponding to factor X1. The reaction was subsequently heated to the designated temperature X2. After X3 h, the reaction mixture was filtered to obtain the crude product.

Purification, drying, and thin-layer chromatography (TLC) analysis were carried out following the procedures previously described.

2.4 Data Analysis

The influence of independent variables on the output was evaluated using ANOVA, with all experiments conducted in triplicate to ensure statistical reliability. Model validity was assessed through the *p*-value, coefficient of determination (R2), adjusted R2, and Adequate Precision. Based on these parameters, Design Expert software generated a regression equation and identified optimal synthesis conditions using the highest desirability value.

2.5 Identification of Characteristics and Structure of the Optimized Product

The obtained product was evaluated based on its organoleptic properties, solubility, and melting point. Its structure was determined using UV-Vis spectroscopy, IR spectroscopy, and both ^{1}H-NMR and ^{13}C-NMR analyses.

3 Results

3.1 Results of the Preliminary Synthesis

Based on the molar ratio of reactants, the theoretical yield corresponded to 0.01 mol of product. The preliminary synthesis was conducted at a molar ratio of Thiourea to Ethyl acetoacetate of 1:1, at a reaction temperature of 70 °C for 3 h. Under these conditions, a total of 0.6566 g of 2-amino-4-methyl-5-ethoxycarbonyl thiazole was obtained, corresponding to a reaction yield of 22.80%.

The reaction conditions typically applied in traditional thiazole syntheses are not fully optimized and often fail to deliver the expected yields. This is mainly due to limitations in controlling and adjusting essential parameters such as temperature, reaction time, and reactant molar ratios. As a result, conventional methods frequently encounter challenges in maximizing efficiency, leading to low product recovery and increased resource consumption in terms of time and cost.

3.2 Thin-layer Chromatography

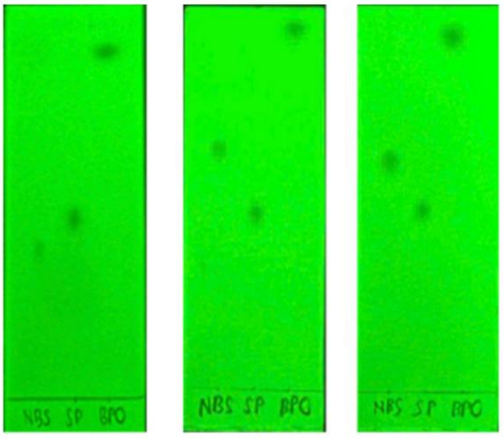


Figure 2 Thin-layer chromatography results
(a) n-hexane : acetone = 6:4; (b) toluene : acetone = 2:1; (c) toluene : ethyl acetate = 1:1

The TLC plate contained three spots arranged from left to right: N-bromosuccinimide (NBS), the

synthesized product (SP), and benzoyl peroxide (BPO), each dissolved in chloroform.

Detection was performed under UV light at 254 nm (Figure 2).

Observation: the chromatogram showed that the product appeared as a single spot with an Rf value distinct from those of the starting materials across all three solvent systems. This indicates structural differences and demonstrates that the purified product is of acceptable purity. The same solvent systems were subsequently applied to analyze samples obtained from the CCD design and the final optimization experiments.

The synthesized product (SP): ^{1}H-NMR (500 MHz, chloroform-d) δ (ppm) 7.29 (s, 2H), 4.31-4.26 (m, 2H), 2.55 (s, 3H), 1.36-1.34 (t, J = 7, 3H); ^{13}C-NMR (125 MHz, chloroform-d) δ (ppm) 169.36; 162.55; 158.95; 111.37; 60.60; 17.21 and 14.41.

3.4 Optimization of The Synthesis of 2-amino-4-methyl-5-ethoxycarbonyl Thiazole Using *RSM*

3.4.1 Model Development and Model Adequacy

To assess the significance of each coefficient in the model, *F*-values and *p*-values were determined using Design Expert 11. Table 2 presents the analysis of variance (ANOVA) results along with the corresponding statistical parameters for each factor.

3.3 Nuclear Magnetic Resonance Spectrum

Table 2 ANOVA Analysis for the Regression Model

Source	*Sum of Squares*	*Mean Square*	*F*-value	*p*-value	Fit Statistics
Model	3167.9	351.99	22.63	**< 0.0001***	
A – Mole ratio of Thiourea : EAA	117.64	117.64	7.56	0.0205	
B – Temperature	698.47	698.47	44.91	**< 0.0001***	
C – Time	68.41	68.41	4.4	0.0624	
AB	15.9	15.9	1.02	0.3357	
AC	0.0041	0.0041	0.0003	0.9874	
BC	0.98	0.98	0.063	0.8069	
A^2	1209.46	1209.46	77.77	**< 0.0001***	
B^2	1016.55	1016.55	65.36	**< 0.0001***	
C^2	453.18	453.18	29.14	0.0003	
Residual	155.52	15.55			
*Indicates the factors with statistically significant *p*-values					

SD = 3.94
M = 35.02
C.V.% = 11.26
R^2 = 0.9532
R^2 (adj.) = 0.9111
AP = 13.3659

The ANOVA for the quadratic model demonstrated high statistical significance (*F*-value = 22.63), with only a 0.01% probability of noise occurrence. Among individual main factors, factor B (temperature, *p*-value < 0.0001) was the most influential variable by directly altering reaction kinetics and molecular collision rates. Factor A (molar ratio of Thiourea to EAA) significantly impacted yield with a *p*-value of 0.0205 (< 0.05) due to its role in molecular contact. Factor C (time) had a *p*-value of 0.0624 (> 0.05), which was not statistically significant at the 95% confidence level, though a potential effect can be inferred at a lower 90% confidence level.

Regarding the interaction terms (AB, AC, and BC), all *p*-values exceeded 0.3, indicating no statistically significant interaction among the variables. This implies that each factor affected the yield independently without notable synergistic or antagonistic effects, simplifying process optimization by allowing individual factor adjustments without complex interaction concerns. Conversely, the quadratic terms (A^2, B^2, and C^2) were all highly significant ($p < 0.001$), confirming a clear non-linear relationship between reaction yield and the investigated factors and justifying the necessity of a quadratic model over a linear one.

The coefficient of determination (R^2) was 0.9532, indicating that 95.32% of experimental variability was explained by the model, well above the 0.75 threshold. Additionally, the adequate precision (AP) reached 13.37, exceeding the minimum threshold of 4 required for navigating the design space, while the coefficient of variation (CV) remained low at 11.26% (below the 20% limit), reflecting high experimental precision and limited random error.

RSM with a five-level (−α, +α, 0, +1, −1) *CCD* was applied to model the relationships among the three main factors: molar ratio of reactants (Thiourea: EAA), reaction temperature, and reaction time. The experimental results from the 20 runs generated by Design-Expert 11 showed that the reaction yield ranged from a minimum of 15.78% (run 20) to a maximum of 51.36% (run 12) (Table 3).

Table 3 Experimental and Predicted Reaction Yields from the 20 *RSM*-Designed Experiments

RUN	A	B	C	Yield (%)		
				Actual value	***Predicted value***	***Residual***
1	1.25	80	6	49.20	50.85	−1.65
2	1	70	3	34.04	35.14	−1.1
3	1.67	80	6	36.30	29.88	6.42
4	1.25	80	6	51.08	50.85	0.2341
5	1.25	63	6	41.33	39.12	2.21
6	1.5	90	3	22.81	27.44	−4.63
7	1.25	80	6	50.47	50.85	−0.3759
8	1.5	70	3	33.63	38.23	−4.6
9	1	70	9	33.99	31.4	2.59
10	1.25	80	11.04	29.09	31.23	−2.14
11	1	90	9	16.13	13.57	2.56
12	1.25	80	6	51.36	50.85	0.5141
13	1.25	80	0.96	43.80	38.76	5.04
14	1.25	80	6	51.20	50.85	0.3541
15	1.25	80	6	51.25	50.85	0.4041
16	1	90	3	18.91	18.71	0.2026
17	1.5	70	9	32.16	34.41	−2.25
18	1.5	90	9	21.27	22.22	−0.9515
19	0.83	80	6	16.50	20.02	−3.52
20	1.25	97	6	15.78	15.08	0.6962

Several additional diagnostic tools were also employed to evaluate the model's suitability. The normal probability plot of residuals demonstrated good agreement between the actual residual distribution and the theoretical normal distribution, as the data points closely followed the diagonal line. Meanwhile, the plot of residuals versus run order confirmed randomness and independence with residuals distributed randomly around zero and showing no discernible patterns, repetition, or systematic structure, thereby supporting the assumptions of independence and randomness.

3.4.2 Optimization of Reaction Yield

The relationship between the reaction yield and the independent variables A, B and C, as generated by Design-Expert 11, is expressed by the final equation in terms of actual factors as follows:

$$\text{Yield} = -641.7758 + 333.83006A + 12.10889B + 7.71325C + 0.564AB - 0.03AC - 0.011667BC - 146.81009A^2 - 0.084121B^2 - 0.084121C^2 \quad (1)$$

where A is the Thiourea to EAA molar ratio, B is the reaction temperature (°C), and C is the reaction time (h). Positive coefficients for A, B, C, and AB indicate that increasing these parameters enhances yield.

Conversely, negative coefficients for the interaction terms (AC, BC) and quadratic terms (A^2, B^2, C^2) correspond to a decline in yield. These negative quadratic terms demonstrate response surface curvature, confirming that excessive factor levels diminish reaction efficiency.

3.4.3 Verification and Evaluation of Model Compatibility

Design-Expert software generated a predictive model with optimized parameters as presented in Table 4. According to this predictive model, the estimated reaction yield under the suggested optimal conditions is 51.6933%. The probability of achieving the desired outcomes at the proposed optimum is 0.919, corresponding to 91.9%. However, to ensure practicality and compatibility with experimental conditions, the model was validated using adjusted values closely aligned with the predicted factors, specifically: a Thiourea : EAA molar ratio of 1.23, a reaction temperature of 77 °C, and a reaction time of 5.75 h.

The experiment was performed six times, and the mean value was used to assess the deviation. The percent difference was calculated according to the equation:

$$\Delta\,(\%) = \frac{|Y_{experimental} - Y_{predicted}|}{Y_{predicted}} \times 100 \quad (2)$$

An error of less than 5% combined with a desirability value of 0.919 indicated that the model parameters were consistent with the experimental results.

Table 4 Results of Six Experimental Runs Performed under the Optimized Conditions

Run	Factor			Yield (%)
	A	B	C	
1	1.23	77	5.75	48.95
2	1.23	77	5.75	51.82
3	1.23	77	5.75	49.21
4	1.23	77	5.75	50.33
5	1.23	77	5.75	52.14
6	1.23	77	5.75	52.57
Mean	**50.84**			

Table 5 Error Analysis Comparing Experimental Yields with Predicted Yields from Design-Expert 11

	Factor			Yield (%)
	A	B	C	
Predicted value	1.229	77.136	5.751	51.6933
Actual value	1.23	77	5.75	50.84
Residual				1.65

The optimized 2-amino-4-methyl-5-ethoxycarbonyl thiazole product appeared as white needle-like crystals, odorless, soluble in methanol, chloroform, and several other organic solvents, and insoluble in water. The melting point was (185-186) °C. UV-Vis (nm) λ_{max} = 351.0; 297.5; IR (cm^{-1}): 3364.00 (ν N-H), 1504.54 (ν C=C), 1273.07 (υ C-N), 756.13 (δ C-H); ^{1}H-NMR (500 MHz, chloroform-d) δ (ppm) 7.29 (s, 2H), 4.31-4.26 (m, 2H), 2.55 (s, 3H), 1.36-1.34 (t, J = 7, 3H); ^{13}C-NMR (125 MHz, chloroform-d) δ (ppm) 169.36; 162.55; 158.95; 111.37; 60.60; 17.21 and 14.41.

4 Discussion

The *RSM* optimization of 2-amino-4-methyl-5-ethoxycarbonyl thiazole synthesis established a quadratic regression model defining the non-linear effects of the Thiourea : Ethyl acetoacetate molar ratio, temperature, and time on reaction yield. While the molar ratio positively influenced yield, temperature and time required moderate control. The absence of significant factor interactions within the studied range simplifies optimization and supports robust process design.

Compared with conventional Hantzsch, Gabriel, and Cook-Heilbron routes – which suffer from fixed conditions, reaction severity, purification issues, and low yields (18-20)% − the *RSM*-based approach provides a simple, cost-effective, and scalable framework. *RSM* reduced the experimental burden relative to one-factor-at-a-time methods while enhancing predictive modeling accuracy [10]. Overall, these findings offer a solid experimental foundation for optimizing thiazole synthesis in antimicrobial, antifungal, and pharmaceutical applications.

5 Conclusion

This study successfully optimized the synthesis of 2-amino-4-methyl-5-ethoxycarbonyl thiazole using *RSM* with a *CCD*. The quadratic model accurately captured the non-linear effects of the Thiourea : Ethyl acetoacetate molar ratio, reaction temperature, and reaction time, predicting optimal conditions (molar ratio 1.23, 77 °C, and 5.75 h) that achieved a 50.84% yield. Product purity was confirmed by UV-Vis, NMR spectroscopy, and TLC analyses. By improving process stability and yield, this optimized protocol reduced reagent consumption and experimental repetition in laboratory-scale thiazole synthesis. These findings offered a practical foundation for medicinal chemistry efforts against antimicrobial resistance, with future research focusing on process scalability, extension to related derivatives, and biological evaluation.

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Tối ưu hóa quy trình tổng hợp 2-amino-4-methyl-5-ethoxycarbonyl thiazol

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Tóm tắt Sự gia tăng tình trạng kháng kháng sinh trên toàn cầu đang trở thành mối đe dọa lớn đối với sức khỏe cộng đồng, làm nổi bật nhu cầu phát triển các tác nhân kháng khuẩn mới. Thiazole được quan tâm nhờ tiềm năng kháng khuẩn, kháng nấm và ứng dụng trong dược phẩm. Tuy nhiên, các phương pháp tổng hợp thiazole truyền thống, như phương pháp Hantzsch (1887), thường chỉ đạt hiệu suất (18-20) %, trong khi các chiến lược tối ưu hóa còn hạn chế. Thử nghiệm ban đầu trong điều kiện thông thường chỉ đạt hiệu suất 22,80 %. Nghiên cứu nhằm tối ưu hóa quá trình tổng hợp 2-amino-4-methyl-5-ethoxycarbonyl thiazole bằng phương pháp bề mặt đáp ứng (RSM). Ba yếu tố gồm tỷ lệ mol thiourea/ethyl acetoacetate, nhiệt độ và thời gian phản ứng được khảo sát theo thiết kế hợp thành trung tâm (CCD). Sản phẩm được tinh chế bằng kết tinh dung môi và đặc trưng bằng UV-Vis, IR, ^{1}H-NMR và ^{13}C-NMR. Ở điều kiện tối ưu (tỷ lệ mol 1,23; 77 °C; 5,75 giờ), hiệu suất đạt (50,84 ± 1,65) %. Kết quả cho thấy RSM có hiệu quả trong việc cải thiện hiệu suất tổng hợp thiazole.

Từ khóa Tổng hợp thiazol; tối ưu hóa; phương pháp đáp ứng bề mặt.