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OPTIMIZATION OF NITRATION OF 3-HYDROXYPYRAZINE-2-CARBOXAMIDE TO 3-HYDROXY-6-NITROPYRAZINE-2-CARBOXAMIDE

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ABSTRACT

This study focuses on optimizing the synthesis of 3-hydroxy-6-nitropyrazine-2-carboxamide, a critical intermediate in producing various pyrazine-based pharmaceuticals. The compound is synthesized through the nitration of 3-hydroxypyrazine-2-carboxamide, employing sulfuric acid (H_2SO_4) and potassium nitrate (KNO_3) as reagents. The research aimed to refine the synthesis process to enhance yield purity for pharmaceutical applications. The optimization entailed adjusting the reagents' composition and solvents, specifically the ratio of substrate to KNO_3, the volume of H_2SO_4 used per gram of substrate, and the temperatures for both the reaction and product precipitation. Optimal results were observed at a substrate-to-KNO_3 ratio of 1:2, with 12 mL of H_2SO_4 per gram of substrate. The reaction temperature was 50°, and precipitation occurred effectively at 0°C. This optimized method significantly improved the yield and purity of the compound. The process demonstrated excellent repeatability, with yields ranging from 77% to 80%, a considerable increase from the 48% yield reported in previous studies. The molecular structure of the synthesized compound was confirmed through comprehensive spectroscopic analyses, including ^{1}H NMR, ^{13}C NMR, and High-Resolution Electrospray Ionization Time-of-Flight Mass Spectrometry (HRESI-TOF-MS). This research represents a significant advancement in synthesizing 3-hydroxy-6-nitropyrazine-2-carboxamide, offering a more efficient and reliable method for producing this key pharmaceutical intermediate. The improved synthesis process ensures higher yields and maintains the purity required for pharmaceutical applications, thereby contributing to the efficient development of pyrazine-based drug compounds.

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INTRODUCTION

Pyrazines, a class of heterocyclic aromatic compounds, are prevalent in nature, appearing in diverse forms, including alkylpyrazines [1] and methoxypyrazines [2]. These compounds are identified in a wide range of biological sources. For instance, alkylpyrazines have been detected in staple vegetables like potatoes [3] and tomatoes [4], adding to their distinct flavors and aromas. In the herbaceous realm, parsley [5] is another example where pyrazines contribute to its characteristic taste and scent. The presence of pyrazines extends beyond the plant kingdom,

with various types being identified in insects such as bees [6] and flies [7]. These findings suggest that pyrazines play a significant role in the chemical ecology of these creatures, possibly influencing their communication and interaction within ecosystems. The role of pyrazines is not limited to their natural occurrence in flora and fauna. Microorganisms also synthesize and degrade these compounds as part of their metabolic processes [8]. This biological synthesis and degradation by microorganisms highlight the ecological importance of pyrazines. Their presence and transformations within microbial communities can influence various environmental processes, including soil nutrient cycling and plant-microbe interactions. The study of pyrazines in microorganisms can thus offer insights into their ecological functions and potential applications in biotechnology, further underlining the multifaceted nature of these aromatic compounds in the natural world.

Alkylpyrazines and methoxypyrazines are known for their potent aroma and are widely used as flavoring agents in various products [9]. Despite their significant sensory impact, these pyrazine compounds are present in very minute quantities in natural source stance; the concentration of pyrazines in a typical beet is a mere 0.01% of its total mass [10]. This minuscule proportion highlights a considerable gap between the natural availability of these compounds and the growing industrial demand for them. Consequently, there is a substantial reliance on chemical synthesis to meet the requirements of various industries, particularly in the food and flavor sector. Synthesis of pyrazines through chemical processes addresses the demand and allows for the creation of a range of pyrazine derivatives, which can be tailored to specific flavoring needs. This approach ensures a steady and scalable supply of these aromatic compounds, which are essential for the continuous development and diversification of flavors in the food industry.

Pyrazine derivatives have expanded their utility beyond the fragrance and flavor industry, finding diverse applications in fields such as dye production, organic semiconductor materials, complex compound ligands, and pharmaceuticals [11]. In medicine, the pyrazine ring plays a crucial role in exhibiting a variety of biological activities [12], underlining its significance in drug development. Notably, several drugs with a pyrazine framework have been developed to treat a range of conditions. These include Acipimox, utilized in diabetes management [13]; Zibotentan, a cancer medication [14]; Pyrazinamide, an antitubercular agent [15]; and Favipiravir (Avigan), known for its antiviral properties [16]. The versatility of pyrazine derivatives in drug design underscores their potential to address various health challenges, demonstrating their critical role in advancing pharmaceutical sciences.

Nitration, a pivotal process in organic synthesis, is widely employed in preparing nitroaromatic compounds [17], including heterocyclic aromatics like pyrazines. Nitropyrazines are particularly significant due to their ability to be transformed into aminopyrazines, which subsequently can be functionalized to yield a range of aminopyrazine derivatives. Notably, several aminopyrazine derivatives exhibit bioactivity as tyrosine kinase inhibitors, highlighting their potential in pharmaceutical applications. For instance, 3-cyclobutyl-1-(4-phenoxyphenyl)imidazo[1,5-

a]pyrazin-8-amine acts as an ACK1 kinase inhibitor [18], while (R)-5-((1-amino-3-methyl-1-oxobutan-2-yl)amino)-3-(quinoline-3-ylamino) pyrazine-2-carboxamide serves as a Syk kinase inhibitor [19]. This bioactivity renders aminopyrazine derivatives as promising candidates for anticancer drug development; thenitration of pyrazine, a critical intermediate step in synthesizing these aminopyrazine derivatives, is of utmost importance. Developing an efficient pyrazine nitration method is essential to ensure the production of nitropyrazine in adequate quantities and purity, thereby facilitating the advancement of novel therapy; Zibotentan, a cancer medication [14]; Pyrazinamide, an antitubercular agent [15]; and producing 3-hydroxy-6-nitropyrazine-2-carboxamide is crucial due to the compound's significance as an intermediate in various medicinal formulations. Previous research employing KNO_3 and H_2SO_4 for this nitration resulted in a yield of only 48%, which is relatively low [20]. Given the compound's importance in the pharmaceutical industry, enhancing the yield is essential to meet the increasing demand for this intermediate. Therefore, methodological advancements and optimization of reaction conditions are needed to increase the yield and ensure adequate and efficient production of 3-hydroxy-6-nitropyrazine-2-carboxamide for its subsequent application in drug synthesis.

METHODS

1. Equipment and Instruments

The equipment consists of various types of glassware commonly used in chemical laboratories, Buchner filters, and BUCHI-R-114 rotary evaporators. Thin layer chromatography (TLC) was carried out on a 1.25 mm Merck Kieselgel 60 F254 silica gel coated aluminum plate and visualization using an SSC-5410 UV-Vis detector working at λ=254nm. The TLC and the appropriate detectors are suitable for identifying aromatic compounds such as pyrazine. The molecular structures of the synthesized compounds were determined using Agilent Technologies NMR Variant Unity INOVA-500 MHz (^{1}H 500 MHz, ^{13}C 125 MHz) at 25^{o}C and Waters LCT Premier XE ESI-TOF (Electron Spray Ionization-Time of Flight) mass spectroscopy. The chemicals used in the synthesis were obtained from Merck, Sigma-Aldrich consisting of 3-hydroxypyrazine-2-carboxamide 98%, potassium nitrate (KNO_3) for analysis, and sulfuric acid (H_2SO_4) 95-97% for analysis.

2. Preparation of Reagents

This research utilized high-grade chemicals obtained from Merck and Sigma-Aldrich, ensuring consistency and reliability in the synthesis process. The primary chemicals used were 3-hydroxypyrazine-2-carboxamide with a purity of 98%, potassium nitrate (KNO_3) for analytical purposes, and sulfuric acid (H_2SO_4) at a 95-97% concentration. The precision in selecting these reagents was crucial for achieving the desired chemical reactions and ensuring the high purity of the synthesized compounds. Each reagent played a specific role in the reaction process, with potassium nitrate as the nitration agent and sulfuric acid as a catalyst and solvent.

3. Synthesis Steps

The synthesis of 3-hydroxy-6-nitropyrazine-2-carboxamide followed a meticulously planned procedure [20] (**Figure 1**). Initially, 1.0 gram (7 mmol) of 3-hydroxypyrazine-2-carboxamide was dissolved

in 6 mL of concentrated sulfuric acid, with careful temperature control using ice cooling to prevent overheating. To this solution, 1.4 g (14 mmol) of potassium nitrate was gradually added. The reaction mixture was then stirred at 40^{o}C for four hours, allowing the nitration process to occur. Post-reaction, the mixture was carefully poured into 60 mL of water, inducing the formation of a precipitate. This precipitate was then isolated using filtration and dried, yielding the desired nitropyrazine compound.

4. Optimization Techniques

Optimization of the synthesis involved adjusting key variables such as the composition of reagents and solvents (KNO_3 and H_2SO_4), the reaction temperature, and the temperature at which the product was precipitated. These factors were systematically varied to identify the optimal conditions that would yield the highest quantity and purity of the final product. Through this process, the research aimed to enhance the synthesis's efficiency, improving yield and purity compared to previous methods.

5. Analytical Methods

The synthesized compounds were characterized using advanced analytical techniques to confirm their molecular structures. Nuclear Magnetic Resonance (NMR) spectroscopy was performed using an Agilent Technologies NMR Variant Unity INOVA-500 MHz instrument, capable of analyzing both hydrogen (^{1}H at 500 MHz) and carbon (^{13}C at 125 MHz) nuclei. Additionally, Mass Spectroscopy was conducted using Waters LCT Premier XE ESI-TOF, providing detailed mass spectra and confirming the molecular weight of the synthesized compounds. Thin Layer Chromatography (TLC) was employed for preliminary compound identification and purity assessment. The TLC process involved a Merck Kieselgel 60 F254 silica gel-coated aluminum plate, with compound detection carried out using an SSC-5410 UV-Vis detector operating at a wavelength of 254 nm, a method particularly suited for aromatic compounds like pyrazine.

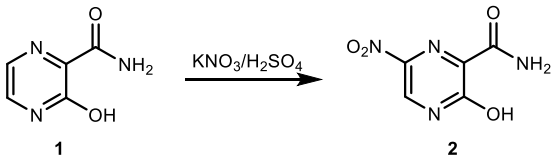


Figure 1. Synthesis of 3-hydroxy-6-nitropyrazine-2-carboxamide (**2**) [20].

RESULTS AND DISCUSSION

In the study, a yellowish-white solid product weighing 0.353 g, corresponding to a yield of 27.4%, was successfully synthesized. Advanced spectroscopic analyses rigorously established this product's chemical identity and structural integrity, particularly focusing on ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy. The NMR data played a pivotal role in confirming the molecular composition and verifying the nitration process of the pyrazine derivative. The ^{1}H NMR spectrum offered critical insights into the structural changes resulting from the reaction. A shift was observed in the proton signal, moving to δ_H 8.9 ppm at the H-5 position. The proton at this position experiences an upfield shift, becoming deshielded due to introducing the nitro group at the 6th position.

This deshielding effect is a direct consequence of the electron-withdrawing nature of the nitro group, which alters the electronic environment around the hydrogen atom at the H-5 position [21]. The comparison

between the substrate's initial ^{1}H NMR spectrum and the product's spectrum reveals substantial differences, providing conclusive evidence of the chemical transformation. This observation is crucial as it confirms the successful nitration reaction and ensures the specific position where the nitration occurred within the pyrazine ring (Figure 2). These spectroscopic findings, particularly the shift in the proton signal and the resulting deshielding effect, align well with the expected outcomes of the nitration process, thereby validating the synthesis of the targeted 3-hydroxy-6-nitropyrazine-2-carboxamide compound.

The synthesized product's ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy data provided essential insights into its molecular structure. According to the data, the product contains four hydrogen and five carbon atoms. These findings are consistent with the expected structure of 3-hydroxy-6-nitropyrazine-2-carboxamide. The peaks observed in the ^{1}H NMR spectrum reflect the hydrogen atoms' environments in the molecule. The four hydrogen atoms in the product give rise to distinct peaks, each corresponding to a specific proton environment in the pyrazine ring structure. These peaks' chemical shifts and splitting patterns provide valuable information about the protons' electronic and spatial environments, confirming the successful incorporation of the nitro group at the desired position on the pyrazine ring. Similarly, the ^{13}C NMR spectrum reveals the carbon framework of the molecule. The five carbon atoms contribute to their respective peaks, with each peak providing insights into the electronic environments of the carbons, including those affected by the nitration process. The chemical shifts observed in the ^{13}C NMR spectrum align with the theoretical predictions and existing literature values for similar compounds, further validating the structural integrity of the synthesized product. The agreement of the NMR data with the literature values [20,22] confirms the successful synthesis of 3-hydroxy-6-nitropyrazine-2-carboxamide and ensures the accuracy and reliability of the analytical methods employed. This correlation is critical for validating the compound's molecular structure and the effectiveness of the synthesis process (**Table 1**).

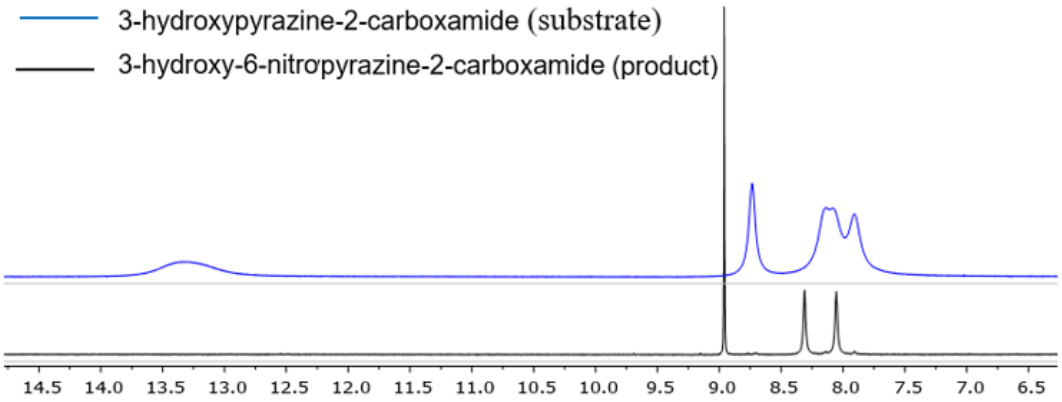
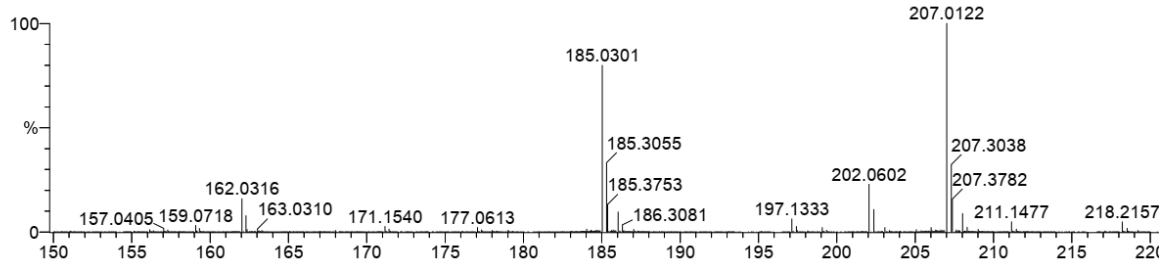


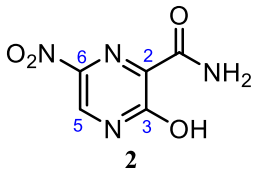
Figure 2. Comparison of ^{1}H NMR signals between substrate and product



Minimum:				-1.5			
Maximum:		5.0	10.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
185.0301	185.0311	-1.0	-5.4	5.5	621.5	0.8	C5 H5 N4 O4

Figure 3. HRESI-TOF MS spectra of **2**

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of **2**



2

Position	**2**		**2* [20]**	
	δ_H (*mult., J in* Hz)	δ_C	δ_H (*mult., J* in Hz)	δ_C
2	-	142.9	-	142.5
3	-	163.2	-	163.1
5	8.96 (*s*)	138.2	8.97 (*s*)	138.2
6	-	133.7	-	133.8
-OH	-	-	12.00 – 15.00 (*bs*)	-
-CO-	-	156.4	-	156.5
$-NH_2$	8.06 (*s*)	-	8.06 (*s*)	-
	8.31 (*s*)	-	8.32 (*s*)	-

The molecular structure of the synthesized compound, referred to as **2**, was further confirmed through high-resolution mass spectrometry (HRMS) using Electrospray Ionization - Time of Flight - Mass Spectrometry (ESI-TOF-MS). The mass spectrum provided crucial evidence supporting the successful synthesis of the compound [23]. The HRMS data revealed a $[M+H]^+$ ion mass peak at m/z 185.0301 and a $[M+Na]^+$ ion at m/z 207.0122. These mass-to-charge ratios corresponded closely to the theoretical values calculated for the molecular ion of 3-hydroxy-6-nitropyrazine-2-carboxamide ($C_5H_4N_4O_4$), which are 185.0311 (for $[M+H]^+$) and 207.0125 (for $[M+Na]^+$) (Figure 3). The close agreement between the observed and calculated mass values provided strong evidence that the compound synthesized was indeed 3-hydroxy-6-nitropyrazine-2-carboxamide, referred to as compound **2** in this study [20,22].

The synthesis yield, however, was only 27.4%, which is lower than the yield reported in previous studies [20]. This lower yield is a matter of concern, especially considering the significance of compound **2** as an intermediate in the synthesis of various pharmaceutical drugs. The suboptimal yield necessitates further optimization of the synthesis process to improve the yield and overall efficiency. Various parameters of the synthesis process were optimized to enhance the yield, including the composition of reagents and solvents (KNO_3 and

H_2SO_4), the reaction temperature, and the temperature at which the product was precipitated. Optimization of these parameters is crucial to improve the synthesis yield, making the process more efficient and suitable for large-scale production of compound **2** [24], which is pivotal as an initial intermediate in the synthesis of diverse medicinal compounds.

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1. Optimization of reagents and solvents (KNO_3 and H_2SO_4) composition

The optimization of the synthesis of 3-hydroxy-6-nitropyrazine-2-carboxamide involved a systematic variation in the composition of reagents and solvents, specifically potassium nitrate (KNO_3) and sulfuric acid (H_2SO_4). H_2SO_4 and KNO_3 were specifically chosen because they are highly regioselective for nitration process [25]. This process focused on determining the optimal conditions to achieve the highest possible yield of the desired product. This optimization study used 1 gram of 3-hydroxypyrazine-2-carboxamide (**1**) as the substrate. The reaction was conducted at a temperature of 40°C for 4 hours. A key observation from the study was the impact of the volume of H_2SO_4 on the yield. It was noted that as the volume of H_2SO_4 increased, there was a corresponding increase in the product yield (Table 2).

Table 2. The result in the optimization of reagents and solvents (KNO_3 and H_2SO_4) composition

KNO_3 (g)	H_2SO_4 (mL)	Product (**2**) (g)	Yield (%)
1.4	6	0.353	27.4
1.4	9	0.718	56.0
1.4	**12**	**0.796**	**61.8**
2.1	9	0.358	27.8
2.1	12	0.510	39.4

The experimental results revealed that the optimal conditions for the highest yield were achieved when reacting 1 gram (7 mmol) of the substrate (1) with 1.4 grams (14 mmol) of KNO_3 using 12 mL of H_2SO_4. Under these conditions, the nitration process of the pyrazine derivative was most efficient, leading to a significant increase in the yield of the desired nitropyrazine compound. This finding is crucial for synthesizing 3-hydroxy-6-nitropyrazine-2-carboxamide, providing a more efficient and effective method for producing this compound in greater quantities. The optimized conditions can be applied in larger-scale syntheses, potentially benefiting the pharmaceutical industry, where this compound serves as a valuable intermediate for various drug compounds.

In synthesizing 3-hydroxy-6-nitropyrazine-2-carboxamide, the role of sulfuric acid (H_2SO_4) is multifaceted and crucial. H_2SO_4 serves as a solvent, facilitating the dissolution and interaction of reactants, and as a key player in the nitration process. Its function as a proton donor is particularly vital, as it contributes protons for forming the nitronium ion (NO_2^+), the active agent responsible for introducing the nitro group into the pyrazine ring. This increased availability of H^+ ions from sulfuric acid promotes the generation of more nitronium ions, essential for the nitration reaction [26]. Additionally, H_2SO_4 acts as a dehydrating agent, binding water molecules produced during the reaction. This action is critical in shifting the equilibrium toward the product side, thereby preventing the reverse reaction and promoting the formation of the desired nitro compound. The addition of H_2SO_4, therefore, not only enhances the solubility of reactants but also plays a significant role in

increasing the production of nitronium ions and acting as a dehydrating agent to drive the reaction toward product formation. These roles collectively contribute to an increased yield of the nitration reaction, underscoring the indispensable nature of H_2SO_4 in this synthetic process.

2. Optimization of reaction temperature

The optimization of reaction temperature plays a crucial role in the synthesis of 3-hydroxy-6-nitropyrazine-2-carboxamide. In this process, the reaction temperature was varied between 40°C to 60°C to determine the optimal condition for achieving the highest yield. The experimental setup involved reacting 1 gram (7 mmol) of the substrate (1) with 1.4 grams (14 mmol) of potassium nitrate (KNO_3) in the presence of 12 mL of sulfuric acid (H_2SO_4) over a duration of 4 hours. The results of the temperature optimization revealed that conducting the reaction at 50°C yielded the best results (as indicated in Table 3). This optimal temperature is significant because it facilitates reaching the necessary activation energy for the reaction. Activation energy is the minimum energy required to initiate a chemical reaction [27]. At the optimal temperature of 50°C, the reaction system gains sufficient energy to overcome this threshold, allowing the reactants to transform into products efficiently.

Table 3. The result in the optimization of reaction temperature

Temperature (^{0}C)	Product (**2**) (g)	Yield (%)
40	0,796	61,8
50	**0,947**	**73,5**
60	0,868	67,4

A higher temperature typically accelerates the rate of a chemical reaction by providing more kinetic energy to the reactant molecules, increasing their movement and the frequency of collisions. However, exceeding the optimal temperature can lead to undesirable side reactions or decomposition of the reactants or products, thereby reducing the yield. On the other hand, a temperature lower than optimal may need to provide more energy for the reactants to reach the activation energy, resulting in a slower reaction rate and lower yield. Therefore, maintaining the reaction at 50°C ensures the reactants have adequate energy for effective collisions and transformations, yielding the desired nitropyrazine derivative better. This temperature optimization is crucial in achieving efficient and economical synthesis of 3-hydroxy-6-nitropyrazine-2-carboxamide.

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3. Optimization of product precipitation temperature

The product precipitation temperature is a critical parameter in the synthesis of 3-hydroxy-6-nitropyrazine-2-carboxamide, impacting the yield and purity of the final product [28]. This temperature refers to the temperature of the water (H_2O) into which the reaction mixture is poured to induce precipitation of the synthesized compound. In the experimental setup for optimizing the product precipitation temperature, 1 gram (7 mmol) of the substrate (1) was reacted with 1.4 grams (14 mmol) of potassium nitrate (KNO_3) using 12 mL of sulfuric acid (H_2SO_4). This reaction was carried out for 4 hours at an optimized temperature of 50°C. The mixture was poured into 60 mL of H_2O following the reaction to precipitate the product.

The temperature of the water used for this purpose can significantly influence the formation and characteristics of the precipitate. For instance, pouring the reaction mixture into

cold water can lead to rapid cooling, resulting in a higher precipitate yield and possibly smaller particle sizes due to rapid nucleation. Conversely, using water at a higher temperature may lead to a slower precipitation process, potentially affecting the crystallinity and purity of the product. The choice of precipitation temperature can also affect the solubility of the product in water. Some compounds might be less soluble in cold water, enhancing their precipitation, while others might require warmer temperatures to precipitate effectively. Therefore, determining the optimal precipitation temperature is essential to maximize yield and ensure the desired physical and chemical properties of the synthesized 3-hydroxy-6-nitropyrazine-2-carboxamide

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Table 4. The result in the optimization of product precipitation temperature

H_2O temperature (°C)	Product (**2**) (g)	Yield (%)
Room temperature	0.899	69.8
Room temperature	0.868	67.4
0	**1.025**	**79.6**
0	**0.972**	**75.5**

The influence of the product precipitation temperature on the synthesis of 3-hydroxy-6-nitropyrazine-2-carboxamide was significant, as indicated by the results presented in Table 4. The experiment involved reacting 1 gram (7 mmol) of the substrate (1) with 1.4 grams (14 mmol) of KNO_3 using 12 mL of H2SO4 for 4 hours at a temperature of 50°C. After the reaction, the mixture was poured into 60 mL of water at different temperatures for precipitation. The findings showed that when the reaction mixture was poured into water at a lower temperature, specifically 0°C, it led to a higher product yield than at room temperature. This effect can be attributed to lower temperatures enhancing the purity and yield of the synthesized crystals. The decrease in temperature reduces the solubility of the compound in water, resulting in more effective precipitation.

The crystallization temperature and the cooling rate are crucial in determining the properties of the resultant crystals. Rapid cooling at 0°C can facilitate quicker nucleation and crystal formation, thereby improving the yield. The dynamics of crystal formation, including nucleation and growth, are heavily influenced by temperature. At lower temperatures, the reduced kinetic energy of the molecules leads to slower molecular motion, which can favor the formation of more stable and well-defined crystal structures. These results underscore the importance of precipitation temperature in the synthesis process. The optimal yield for 3-hydroxy-6-nitropyrazine-2-carboxamide is achieved by precipitating the reaction mixture in water cooled to 0°C, demonstrating the pivotal role of this parameter in enhancing the effectiveness of chemical synthesis [27].

4. Scalled-up reaction

The optimization of the synthesis of 3-hydroxy-6-nitropyrazine-2-carboxamide (2) from 3-hydroxypyrazine-2-carboxamide (1) was achieved through meticulous adjustment of reagents and solvents (KNO_3 and H_2SO_4) composition, reaction temperature, and product precipitation temperature. The optimal conditions determined were reacting 1 gram (7 mmol) of the substrate (1) with 1.4 grams (14 mmol) of KNO_3, using 12 mL of H_2SO_4, maintaining the reaction temperature at 50°C for 4 hours, followed by pouring the reaction mixture into 60 mL of water cooled to 0°C for

precipitation. This optimized method was then applied to synthesize the compound at increased quantities, as shown in Table 5. The results indicated that under these optimum conditions, and upon scaling up the reaction to 1, 3, 6, and 12 times the original amount, the yield remained relatively consistent, ranging between 77% and 80%. This yield consistency demonstrates the optimized method's scalability and reliability, making it suitable for large-scale or industrial synthesis.

Table 5. Yield of scalled-up reaction

1 (g)	KNO_3 (g)	**1:** KNO_3 (mol)	H_2SO_4 (mL)	Reaction temp. (^oC)	H_2O temp. (^oC)	Time (h)	Yield (%)
1	1.4	1: 2	12	50	0	4	79.6
3	4.2	1: 2	36	50	0	4	78.9
6	8.4	1: 2	72	50	0	4	77.4
12	16.8	1: 2	144	50	0	4	77.1

However, it was observed that there was a slight decrease in yield percentage at larger scales. This could be attributed to temperature distribution and control variations during the scaled-up reactions. As the reaction scale increases, maintaining uniform temperature throughout the mixture becomes more challenging. This issue can be addressed by implementing more precise and controlled temperature management techniques. Enhanced stirring mechanisms and more accurate temperature monitoring could be potential solutions to ensure temperature uniformity, thereby maintaining yield efficiency. Optimizing the synthesis process has been successful, providing a reliable method for producing 3-hydroxy-6-nitropyrazine-2-carboxamide at various scales with consistent yields. This advancement holds significance in producing this intermediate compound, which is essential in the pharmaceutical industry for developing various drug compounds.

CONCLUSION

The synthesis of 3-hydroxy-6-nitropirazine-2-carboxamide (**2**) from 3-hydroxypyrazine-2-carboxamide (**1**) obtained optimum results at a ratio of substrate: KNO_3 = 1: 2, using 12 mL H_2SO_4 for every 1 g of substrate, with the reaction temperature is 50^oC and the product precipitation temperature is 0^oC. The synthesis carried out under the optimum conditions has good repeatability, with the yield in the 77% -80% range. This research has a significant scientific contribution. The synthetic method developed, including obtaining good synthetic yields and repeatability, can be used as a reference in providing access to nitropyrazine and its derivatives. Apart from that, this research also contributes to developing active compounds as drug candidates.

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