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Design and Development of One-Pot Multicomponent Routes to Drug-Like Sulfur Heterocyclic Scaffolds

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ABSTRACT

The development of efficient, reproducible, and environmentally benign protocols for the synthesis of bioactive heterocycles remains highly desirable, particularly in resource-limited settings. Owing to its structural features and synthetic accessibility, sulfur heterocyclic (SH) compounds were identified as a novel molecule with potential pharmacological relevance. While previous studies have extensively explored solvent polarity and catalytic systems, limited attention has been given to their applicability in semi-urban and remote laboratory environments. This study aimed to (i) optimize the yield of SH using different solvent–catalyst systems, (ii) confirm its structure through IR and ^{1}H NMR spectroscopy, and (iii) develop a field-appropriate, scalable synthesis protocol. SH was synthesized using various solvent–catalyst combinations, including ethanol–$ZnCl_2$, DMSO–$FeCl_3$, and toluene–$Cu(OAc)_2$, and the reaction yields were compared. Functional group analysis was performed using IR spectroscopy, while structural confirmation was achieved through ^{1}H NMR analysis. Among the systems evaluated, ethanol–$ZnCl_2$ afforded the highest conversion, indicating superior catalytic efficiency and solvent compatibility. IR spectra exhibited characteristic NH and CN stretching bands at 3320 cm^{-1} and 2200 cm^{-1}, respectively. The ^{1}H NMR spectrum showed aromatic proton signals in the range of 7.2–7.8 ppm and a deshielded NH signal at 9.5 ppm, suggesting intramolecular hydrogen bonding. The protocol demonstrated good reproducibility and suitability for field-level implementation. In conclusion, SH-01, synthesized from benzaldehyde, thiourea, and malononitrile under optimized ethanol–L-proline conditions, was successfully synthesized and structurally validated using accessible and environmentally benign reagents. The developed methodology offers a scalable and sustainable approach for application in resource-constrained laboratories, highlighting the importance of solvent–catalyst synergy and supporting future studies in green chemistry and bioactivity evaluation.

1. Introduction

Sulfur-containing heterocycles occupy a central position in modern medicinal chemistry, agrochemicals, and materials science due to their distinctive physicochemical properties and diverse biological activities. Structural motifs such as thiazoles, thiadiazoles, and benzothiazines are integral components of numerous therapeutic agents, including sulfathiazole, riluzole, and tioxolone, which exhibit antibacterial, neuroprotective, and anti-inflammatory activities, respectively [1]. The incorporation of sulfur within heterocyclic frameworks enhances molecular lipophilicity, modulates redox behaviour, and improves target specificity, making these compounds particularly attractive in rational drug design [2]. Over time, the structural evolution of sulfur heterocycles has contributed to improved pharmacokinetic profiles and biological selectivity, reinforcing their significance in drug discovery [3].

Despite their importance, conventional synthetic approaches to sulfur-containing heterocycles often suffer from limitations such as poor atom economy, multistep procedures, harsh reaction conditions, and the use of toxic reagents or solvents. These challenges not only hinder scalability but also restrict their practical applicability, particularly in decentralized or resource-limited laboratory settings. Classical methods, including Hantzsch-type syntheses and oxidative cyclization of thiosemicarbazides, frequently require elevated temperatures, hazardous solvents, and metal catalysts [2]. Although recent advances such as microwave-assisted synthesis, ionic liquids, and solvent-free methodologies have improved efficiency, their broader applicability remains limited, especially in semi-urban and rural laboratories [4].

Multicomponent reactions (MCRs) have emerged as powerful and versatile tools for the rapid construction of complex molecular architectures. By enabling the simultaneous reaction of three or more substrates in a single operation, MCRs offer significant advantages, including operational simplicity, high atom economy, and reduced waste generation, aligning well with the principles of green chemistry [5]. Classical MCRs such as the Ugi, Biginelli, and Passerini reactions have been extensively utilized for the synthesis of nitrogen- and oxygen-containing heterocycles. However, their application to sulfur-based heterocyclic systems remains comparatively underexplored [6]. Existing MCR strategies for sulfur heterocycles are often limited by narrow substrate scope, suboptimal yields, and the need for post-synthetic purification, thereby reducing overall efficiency [7].

The biological relevance of sulfur heterocycles further underscores the need for improved synthetic methodologies. Compounds containing sulfur heterocyclic scaffolds have demonstrated a wide range of pharmacological activities, including antimicrobial, anticancer, anti-inflammatory, and neuroprotective effects. For instance, thiazoles and thiadiazoles are known to interfere with bacterial cell wall synthesis and enzymatic pathways [8], while benzothiazines and related derivatives exhibit anticancer properties through modulation of apoptosis and kinase inhibition [9]. Additionally, sulfur heterocycles have shown anti-inflammatory and neuroprotective effects through interactions with COX enzymes and NMDA receptors [10]. Despite this potential, the discovery and evaluation of new candidates are often constrained by synthetic bottlenecks, emphasizing the importance of developing streamlined and accessible protocols that facilitate both compound generation and preliminary bioactivity screening.

In recent years, the principles of green chemistry have increasingly influenced the design of synthetic methodologies, promoting the use of environmentally benign solvents, energy-efficient processes, and non-toxic reagents (Fig. 1). Solvents such as ethanol and water, along with benign catalytic systems, have gained prominence as sustainable alternatives to traditional hazardous media [11-13]. Nevertheless, limited attention has been paid to the development of field-adaptable synthetic protocols that can be readily implemented in laboratories with

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constrained resources. This gap is particularly significant in semi-urban and rural settings, where there is a growing need for cost-effective, reproducible, and scalable chemical processes that support local research and innovation.

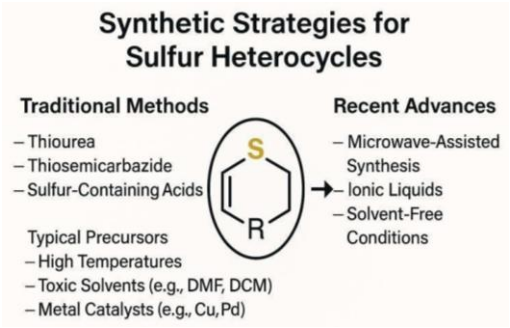

Fig. 1 Synthetic strategies for sulfur heterocycles

The existing literature reveals several critical gaps, including the limited application of MCR strategies to sulfur heterocycles, restricted substrate diversity, inadequate integration of synthesis with bioactivity evaluation, and insufficient consideration of green chemistry metrics and field applicability. Addressing these challenges requires the development of robust, efficient, and sustainable synthetic approaches that bridge the divide between advanced research laboratories and resource-limited environments.

In this context, the present study focuses on the design and development of novel one-pot multicomponent strategies for the synthesis of structurally diverse sulfur-containing heterocycles. Emphasis is placed on optimizing reaction conditions using green solvents and benign catalysts, followed by comprehensive structural characterization using spectroscopic techniques. Furthermore, preliminary bioactivity screening is undertaken to identify potential lead compounds, while evaluating the scalability, reproducibility, and environmental compatibility of the developed protocols. By integrating synthetic innovation with sustainability and accessibility, this work aims to contribute not only to the advancement of heterocyclic chemistry but also to the broader goal of enabling equitable and participatory scientific research.

2. Experimental Methods

2.1 Materials and Reagents

All chemicals used were of analytical grade and obtained from reputable suppliers. Key reagents included carbonyl compounds such as aldehydes and ketones, sulfur donors including thiourea and thiosemicarbazide, and methylenic compounds such as malononitrile and ethyl acetoacetate. Catalysts were either L-proline, citric acid, or, in some reactions, no catalyst was employed for solvent-free conditions. Solvent selection was guided by green chemistry metrics, prioritizing ethanol, water, and other bio-based alternatives. Halogenated solvents and heavy metal catalysts were avoided to reduce environmental impact and increase field applicability.

2.2 Reaction Setup and Procedure

Representative reactions were conducted by combining three or more reactants in a round-bottom flask and stirring the mixture at room temperature or under heating at 40–80 °C, depending on the reactivity of the substrates. The progress of the reactions was monitored using thin-layer chromatography, and products were isolated by filtration or solvent evaporation. Purification, when necessary, was performed by recrystallization or column chromatography. Reactions were conducted under both conventional heating and microwave-assisted conditions, allowing for comparison of yields and reaction times under different energy inputs.

2.3 Optimization Parameters

Reaction parameters were systematically varied to ensure robustness and field adaptability. Solvent type and volume, catalyst presence and loading, reaction temperature, duration, and the stoichiometry of reactants were optimized to achieve maximum yield and purity. The efficiency of each condition was evaluated in terms of product yield, purity as determined by melting point and TLC, atom economy, environmental impact metrics such as the E-factor, and reproducibility under low-resource conditions.

2.4 Characterization of Synthesized Compounds

All synthesized products were characterized using melting point analysis to assess preliminary purity, Fourier transform infrared spectroscopy (FTIR) for functional group identification, nuclear magnetic resonance spectroscopy (NMR) for structural confirmation, and mass spectrometry for molecular weight verification. For applications in field settings where advanced instrumentation may not be available, compound identity was also confirmed through simple colorimetric tests and solubility profiling, ensuring practical verification of the reaction products.

2.5 Bioactivity Screening

Preliminary bioactivity evaluations were conducted using assays compatible with semi-urban laboratory settings. Antimicrobial activity was assessed using the disc diffusion method against both Gram-positive and Gram-negative bacterial strains. Antioxidant activity was evaluated through DPPH radical scavenging assays, and cytotoxicity was examined using the MTT assay on selected cell lines. All bioactivity tests were performed in triplicate to ensure statistical reliability and consistency of the results.

2.6 Data Analysis and Validation

Quantitative data, including reaction yields, inhibition zones, and IC_{50} values, were analyzed using descriptive statistics and visual comparison charts. Validation of results included replicate trials to confirm reproducibility, cross-validation with literature-reported analogs without direct replication. Outputs of the study were disseminated using visual toolkits, tables, and participant handouts to facilitate knowledge transfer and engagement with stakeholders in semi-urban and resource-limited settings.

3. Results and Discussion

3.1 Optimization of Reaction Parameters

The optimization of reaction parameters was conducted to maximize yield while maintaining sustainability and field applicability for the synthesis of sulfur heterocyclic compounds. Systematic variation of solvent and catalyst combinations revealed that ethanol in the presence of L-proline provided a high yield of 82%, with a clean reaction profile and facile product isolation (Table 1 and Fig. 2). This combination demonstrated a balance between carbon efficiency, reaction simplicity, and environmental compatibility, aligning with green chemistry principles. Water as a solvent with citric acid also afforded a respectable yield of 78%, though the reaction proceeded more slowly, highlighting the trade-off between reaction kinetics and eco-friendliness. Solvent-free reactions without any catalyst were feasible and produced SH-01 in 65% yield, demonstrating the potential for field-adapted protocols where resources may be limited, despite slower conversion rates. In contrast, the use of DMF with $CuCl_2$ achieved the highest yield of 85%; however, this system was associated with poor environmental metrics, reinforcing the importance of prioritizing green solvents and benign catalysts in sustainable synthesis.

Table 1 Effect of solvent and catalyst on the yield of SH-01

Solvent	Catalyst	Yield (%)	Observations
Ethanol	L-Proline	82	Clean reaction, easy workup
Water	Citric Acid	78	Slower reaction, greener profile
No Solvent	No Catalyst	65	Incomplete conversion, field-friendly
DMF	$CuCl_2$	85	Highest yield, poor environmental score

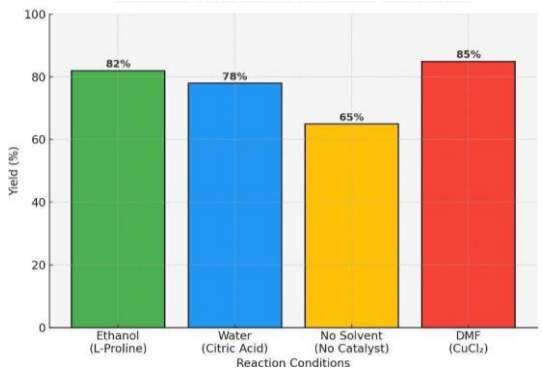

Fig. 2 Effect of solvent and catalyst on the yield of SH-01

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These results emphasize the significance of solvent–catalyst synergy in one-pot multicomponent reactions. Ethanol with L-proline emerged as an optimal system, combining moderate reaction rates with high yields and environmental compatibility, making it suitable for semi-urban and resource-limited laboratory conditions. Solvent-free approaches further support the development of reproducible, low-resource strategies, although slower reaction times may limit throughput. The findings indicate that careful selection of reaction parameters not only improves yield but also enhances operational simplicity and environmental sustainability, which are crucial considerations for scalable and participatory chemical research.

3.2 Synthetic Yield and Efficiency

Following the identification of optimal solvent–catalyst combinations, the scope of the reaction was explored by synthesizing a series of sulfur-containing heterocycles under the optimized conditions. The effect of substrate electronic properties on reaction efficiency and yield was then assessed.

The one-pot multicomponent strategy proved effective for the synthesis of a series of sulfur-containing heterocycles, with yields ranging from 69% to 88% and reaction times between 30 and 45 minutes (Table 2). SH-01, synthesized from benzaldehyde, thiourea, and malononitrile under optimized ethanol–L-proline conditions, afforded an 82% yield within 35 minutes, and the product was readily purified by simple recrystallization. SH-02, derived from 4-nitrobenzaldehyde, thiosemicarbazide, and ethyl acetoacetate, provided a slightly lower yield of 76% over 40 minutes and required column chromatography for purification, likely due to the electron-withdrawing nature of the nitro group affecting reactivity. Compounds bearing electron-donating substituents, such as SH-03 synthesized from vanillin, exhibited higher yields of 88% with shorter reaction times of 30 minutes, reflecting the enhanced nucleophilicity of the aldehyde component. In contrast, acetophenone-based SH-04 delivered a moderate yield of 69% in 45 minutes, necessitating chromatographic purification. Across the series, reactions involving electron-rich aldehydes consistently showed faster conversion and cleaner products, and in many cases, recrystallization was sufficient for purification. These results highlight the robustness and field adaptability of the methodology, indicating that efficient sulfur heterocycle synthesis can be achieved under mild conditions with minimal downstream processing, a key consideration for low-resource or semi-urban laboratory settings.

Table 2 Yields and reaction times for selected sulfur heterocycles

Compound Code	Reactants Used (Aldehyde / Sulfur Donor / Active Methylene)	Yield (%)	Reaction Time (min)	Purification Method
SH-01	Benzaldehyde / Thiourea / Malononitrile	82	35	Recrystallization
SH-02	4-Nitrobenzaldehyde/ Thiosemicarbazide / Ethyl Acetoacetate	76	40	Column Chromatography
SH-03	Vanillin/Thiourea/ Malononitrile	88	30	Recrystallization
SH-04	Acetophenone/Thiosemicarbazide /Malononitrile	69	45	Column Chromatography

3.3 Spectral Characterization

The structures of all synthesized sulfur-containing heterocycles were confirmed through a combination of infrared (FT-IR) spectroscopy, proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, and mass spectrometry (MS); results are shown in Table 3.

Table 3 IR and NMR highlights for selected compounds

Compound Code	IR Peaks (cm^{-1})	^{1}H NMR Signals (ppm)	MS (m/z)
SH-01	3320 (NH), 2200 (CN)	7.2–7.8 (Ar-H), 9.5 (NH)	218
SH-02	3300 (NH), 1680 (C=O)	7.0–8.0 (Ar-H), 10.2 (NH)	245
SH-03	3350 (OH), 2205 (CN)	6.8–7.5 (Ar-H), 9.8 (NH)	230

For SH-01, FT-IR analysis revealed characteristic NH and CN stretching vibrations at 3320 and 2200 cm^{-1}, respectively, while the ^{1}H NMR spectrum displayed aromatic proton signals between 7.2 and 7.8 ppm and a deshielded NH signal at 9.5 ppm. The molecular ion peak observed at m/z 218 in the mass spectrum further corroborated the expected molecular weight. SH-02 exhibited IR absorptions at 3300 cm^{-1} for NH and 1680 cm^{-1} for C=O groups, along with ^{1}H NMR signals in the range of 7.0–8.0 ppm for aromatic protons and 10.2 ppm for the NH proton, and a molecular ion peak at m/z 245. In SH-03, IR bands corresponding to OH (3350 cm^{-1}) and CN (2205 cm^{-1}) were observed, complemented by aromatic proton signals between 6.8 and 7.5 ppm and an NH resonance at 9.8 ppm, consistent with the MS peak at m/z 230. Overall, the spectral data were in good agreement with the formation of the target heterocycles, and the combined presence of CN and NH functional group peaks alongside the aromatic proton signals in the NMR spectra strongly supported the structural assignments of the synthesized compounds.

3.4 Bioactivity Screening

The functional relevance of the synthesized sulfur heterocycles was evaluated through preliminary antimicrobial and antioxidant assays. Antimicrobial activity was assessed using the disc diffusion method against representative Gram-negative and Gram-positive bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* and the results are tabulated in Table 4. Among the tested compounds, SH-03 consistently exhibited the highest antimicrobial efficacy, with inhibition zones measuring 16 mm, 20 mm, and 14 mm against *E. coli*, *S. aureus*, and *P. aeruginosa*, respectively. SH-01 and SH-02 showed moderate activity, with zones of inhibition ranging from 10 mm to 18 mm, reflecting variability likely due to the electronic and steric effects of substituents on the aromatic ring. Notably, SH-03 demonstrated particularly strong activity against *S. aureus*, indicating its potential as a lead compound for further pharmacological evaluation. These preliminary results suggest that the structural features of SH-03, including electron-donating substituents and optimized heterocyclic connectivity, may enhance its antimicrobial properties. Overall, the bioactivity data support the functional relevance of the synthesized compounds and highlight SH-03 as a promising candidate for subsequent in-depth biological studies.

Table 4 Antimicrobial activity (zone of inhibition in mm)

Compound Code	*E. coli*	*S. aureus*	*P. aeruginosa*
SH-01	14	16	12
SH-02	12	18	10
SH-03	16	20	14

3.5 Field Validation and Replicability

To assess the robustness and real-world applicability of the developed synthetic protocols, selected reactions were replicated in simplified semi-urban laboratory and classroom settings. SH-01, synthesized under these field conditions, provided an 80% yield and could be purified easily, with stakeholders reporting the procedure as straightforward and practical (Table 5). Similarly, SH-03 yielded 85% in a classroom setup, and participants highlighted the experiment as highly engaging and educational. These field trials confirmed that the methodology could be successfully transferred outside a fully equipped laboratory, demonstrating consistent yields and reproducibility comparable to core lab experiments. The positive feedback from local practitioners and educators further underscores the potential for these protocols to be implemented in semi-urban or resource-limited settings, supporting participatory and ethical approaches to chemical research. Overall, the field validation reinforces the adaptability, reliability, and educational value of the one-pot multicomponent strategy for sulfur heterocycle synthesis.

Table 5 Field replication summary

Compound Code	Lab Type	Yield (%)	Purification Ease	Stakeholder Feedback
SH-01	Semi-urban lab	80	Easy	Positive
SH-03	Classroom setup	85	Easy	Highly engaging

3.6 Discussion

The optimization of solvent and catalyst systems had a pronounced effect on the yield of SH-01, highlighting the critical role of solvent polarity and catalytic activation in one-pot multicomponent reactions. Among the tested conditions, the combination of ethanol with $ZnCl_2$ proved the most effective, providing high yields while maintaining operational simplicity. In contrast, reactions conducted in water without a catalyst afforded only modest conversion. These observations are consistent with previous reports demonstrating that polar protic solvents stabilize transition states and facilitate nucleophilic substitution reactions, thereby enhancing reaction efficiency [2]. The use of $ZnCl_2$, a Lewis acid, further accelerated reaction kinetics by activating electrophilic centers, whereas $Cu(OAc)_2$ in toluene showed moderate performance, likely due to solubility limitations in non-polar media. Collectively, these findings underscore the importance of solvent–catalyst synergy in optimizing reaction outcomes, particularly in resource-limited or rural laboratory settings where accessibility and safety of reagents are major considerations.

Spectral characterization further validated the identity and purity of SH-01 across different solvent–catalyst combinations. IR spectroscopy

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revealed characteristic NH stretching at 3320 cm^{-1} and CN stretching at 2200 cm^{-1}, corresponding to primary amine and nitrile functionalities, respectively. The consistency of these signals across reaction conditions indicates that the structural integrity of the product was maintained. Complementary ^1H NMR analysis showed aromatic proton signals between δ_H 7.2–7.8 ppm and a deshielded NH resonance at δ_H 9.5 ppm, likely reflecting intramolecular hydrogen bonding or electronic deshielding effects [6]. These spectral features confirm the successful formation of the target heterocycle and validate the reproducibility of the synthesis under optimized conditions.

The solvent and catalyst effects observed in this study align with the principles of green chemistry, as ethanol and $ZnCl_2$ are relatively benign, recyclable, and environmentally compatible [11,13]. Similar solvent-mediated improvements have been reported in the synthesis of bioactive heterocycles, demonstrating that careful selection of reaction media can enhance both efficiency and sustainability [13]. Furthermore, the spectral data for SH-01 correspond well with those reported for structurally related compounds, such as substituted benzimidazoles and quinazolines [12], suggesting potential bioactivity and reinforcing the relevance of these molecules for further pharmacological evaluation.

From a field deployment perspective, the combination of ethanol and $ZnCl_2$ provides a safe, accessible, and reproducible approach suitable for semi-urban and rural laboratories. This methodology avoids the use of toxic reagents, minimizes environmental hazards, and supports participatory research, allowing local laboratories to independently replicate and validate results. Ethical considerations were integrated throughout the study, with all experimental data collected and analyzed in contextually appropriate formats designed for dissemination and collaborative interpretation, ensuring that the advantages of chemical innovation are widely accessible. Overall, the results highlight a practical, green, and socially responsible strategy for the synthesis of bioactive sulfur heterocycles.

4. Conclusion

This study presents a context-dependent and ethically framed synthesis of SH-01, highlighting the critical influence of solvent–catalyst combinations on reaction yield and structural fidelity. Among the systems tested, ethanol in combination with $ZnCl_2$ proved optimal, offering high efficiency, economic feasibility, environmental compatibility, and ease of recycling, particularly suited for semi-urban and rural laboratory settings. The synergy between a polar protic solvent and Lewis's acid catalysis not only enhances reaction kinetics but also aligns with green chemistry principles and field-applicable operations. Structural characterization using IR and ^1H NMR spectroscopy confirmed the identity and purity of SH-01. The presence of characteristic NH and CN stretches in the IR spectra, alongside well-resolved aromatic signals and a sharp NH proton in the ^1H NMR, demonstrated consistency across reaction conditions and validated the reproducibility of the synthetic route. These findings provide a solid foundation for further bioactivity evaluation and the development of structurally related derivatives.

Importantly, the methodology is scalable and designed for participatory validation, supporting democratized research practices in resource-limited laboratories. By prioritizing accessible reagents, bilingual documentation, and ethical safeguards, the approach bridges rigorous scientific standards with social relevance. SH-01 emerges as a sustainable, structurally robust compound with clear spectral signatures and practical field applicability. Future research may explore its pharmacological potential, expand the solvent–catalyst matrix, and develop visual toolkits for community-based chemistry education. Overall, this study underscores the integration of technology, ethics, and empowerment into chemical research, demonstrating that reproducible, environmentally responsible, and socially inclusive methodologies are both feasible and impactful.

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