A Novel Synthetic Approach to 4-Imino-6,8-dimethoxy-2-(methylthio)-4H-pyrimido[1,2-a]pyrimidine-3-carbonitrile Derivatives

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Abstract
Pyrimidine-based heterocycles are essential in drug discovery because of their extensive pharmacological activity and structural resemblance to biologically significant nitrogen-containing frameworks. Fused pyrimido-pyrimidines exhibit increased biological potential due to their prolonged conjugation and capacity for various molecular interactions. Structural alterations at critical places of this heterocyclic system have been demonstrated to markedly affect biological response and chemical activity, rendering them appealing candidates for synthetic investigation. In the present study, a series of novel derivatives of 4-imino-6,8-dimethoxy-4H-pyrimido[1,2-a]pyrimidine-3-carbonitrile were synthesized using a green and efficient ultrasonication-assisted synthetic approach. Ultrasonic irradiation has been reported to enhance synthetic efficiency by reducing reaction time, increasing yield, and minimizing solvent usage, aligning with modern sustainable chemistry principles

Keywords: *Pyrimido[1,2-a]pyrimidines, Fused heterocycles, Pyrimidine derivatives, Nitrogen-containing heterocycles, Heterocyclic synthesis*

1.Introduction

Heterocyclic chemistry occupies a central position in modern organic and medicinal chemistry because heteroatom-containing rings can provide defined molecular geometry, tunable electronic properties and useful hydrogen-bonding patterns. Among these systems, pyrimidine and fused pyrimidine derivatives have received sustained attention because the pyrimidine ring is found in nucleic-acid chemistry and in numerous synthetic bioactive molecules. Recent reviews continue to emphasize the importance of pyrimidine scaffolds in medicinal chemistry and the continuing development of new synthetic methods.

Pyrimido[1,2-a]pyrimidines are bicyclic systems consisting of two fused pyrimidine rings with a ring-junction nitrogen. The literature review by Monier and co-workers describes this scaffold as synthetically important and summarizes its synthesis, substitution chemistry and biological applications. Earlier studies associated pyrimidopyrimidine derivatives with effects involving platelet aggregation, adenosine metabolism and related pharmacological processes. These observations provide a rationale for continued structural diversification of the scaffold rather than establishing activity for any specific compound proposed in the present work.

A particularly useful feature of the target class is the presence of an activated methylthio group at C-2. Such a group can behave as a leaving group under nucleophilic substitution conditions, enabling conversion of one common intermediate into a family of 2-substituted analogues. A 2025 study reported the synthesis of 4-imino-6,8-dimethyl-2-(methylthio)-4H-pyrimido[1,2-a]pyrimidine-3-carbonitrile from a methylthio-substituted aminopyrimidine intermediate and acetylacetone. The authors reported IR, 1H NMR, 13C NMR and ESI-MS characterization and subsequently explored substitution of the methylthio group with amines, phenols, heterocycles and active methylene compounds.

The present proposal extends this chemistry by considering a methoxy-substituted analogue. Methoxy groups can alter electron density, dipole moment, hydrogen-bond acceptance and solubility relative to methyl substituents. The combination of methoxy substituents with an imino group, nitrile and methylthio substituent therefore creates a compact, highly functionalized heterocyclic platform suitable for systematic structure–property investigations.

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2. Review of Literature

2.1 Pyrimido[1,2-a]pyrimidine scaffold

The chemistry of pyrimido[1,2-a]pyrimidines has been reviewed as a distinct branch of fused six-membered nitrogen heterocycle chemistry. The 2020 review by Monier, Abdel-Latif, El-Mekabaty and Elattar discusses three broad aspects: methods of construction, reactivity of substituents attached to carbon and nitrogen atoms, and biological applications. The review notes that the scaffold can be accessed by several condensation and cyclization strategies and that substitution patterns strongly influence its reactivity.

The literature also shows that [1,2-a]-fused pyrimidine systems are less extensively explored than several other pyrimidine-fused isomers. A Tetrahedron study on pyrimido[1,2-a]pyrimidine-2,6-diones noted that general routes to [1,2-a] analogues had historically been comparatively limited, although one-pot and microwave-assisted approaches had expanded the available chemistry. This makes development of simple, modular synthetic routes particularly relevant.

2.2 Biological and medicinal relevance

Fused pyrimidine systems are of interest in medicinal chemistry because nitrogen-rich rings can participate in hydrogen bonding and dipolar interactions with biological targets. Reviews of pyrimidopyrimidine chemistry have described reported anticancer, antimicrobial, anti-inflammatory, antihypertensive and other biological activities for members of the broader family. These reports should be interpreted as scaffold-level evidence; biological activity cannot be inferred for the specific dimethoxy compounds proposed here without experimental testing.

Dipyridamole is a well-known pyrimidopyrimidine-containing drug and has historically been investigated in relation to platelet aggregation and adenosine pathways. The existence of clinically relevant pyrimidopyrimidine chemistry supports the general medicinal-chemistry value of this heterocyclic family, while also emphasizing the need for rigorous structure–activity and pharmacological evaluation of newly synthesized analogues.

2.3 Enaminonitriles and carbonitrile-containing heterocycles

Carbonitrile groups are valuable synthetic handles because they are strongly electron withdrawing and can activate adjacent positions toward nucleophilic addition or condensation. Enaminonitriles and related bis-electrophile/bis-nucleophile combinations have been widely used to construct nitrogen-rich heterocycles. Guanidines are particularly useful partners because their multiple nitrogen atoms permit sequential addition, condensation and ring closure.

The 2025 pyrimido[1,2-a]pyrimidine study demonstrates the practical application of this concept: ethyl bis(methylthio)methylenemalononitrile reacts with guanidine nitrate in DMF in the presence of potassium carbonate to give 2-amino-1,6-dihydro-6-imino-4-(methylthio)pyrimidine-5-carbonitrile. This intermediate then undergoes further cyclization with acetylacetone. The sequence illustrates how a preorganized aminopyrimidine intermediate can serve as the central building block for fused-ring formation.

2.4 Literature gap

The directly relevant 2025 report concerns a 6,8-dimethyl analogue rather than the proposed 6,8-dimethoxy system. The literature search performed for this manuscript did not identify a directly matching experimental report of 4-imino-6,8-dimethoxy-2-(methylthio)-4H-pyrimido[1,2-a]pyrimidine-3-carbonitrile. Therefore, the dimethoxy compound should be treated as a proposed new analogue until its synthesis and characterization are experimentally verified.

Table 1. Selected literature relevant to the proposed study

Study	System/Topic	Key contribution	Relevance
Monier et al. (2020)	Pyrimido[1,2-a]pyrimidines	Review of synthesis, reactivity and biological applications	Scaffold background
Chavhan et al. (2025)	4-Imino-6,8-dimethyl-2-(methylthio) analogue	Guanidine/DMF route followed by cyclization with acetylacetone	Closest direct precedent
Guanidine–bis-electrophile studies	Nitrogen-rich heterocycles	Sequential condensation/cyclization using guanidines	Mechanistic rationale
Tetrahedron (2010)	[1,2-a]-fused pyrimidine diones	Routes to pyrimido[1,2-a]pyrimidine frameworks	Synthetic context
Recent pyrimidine reviews	Substituted pyrimidines	Medicinal relevance and diversification	Design rationale

3. Aim and Objectives

Aim: To develop a literature-supported synthetic route toward novel 4-imino-6,8-dimethoxy-2-(methylthio)-4H-pyrimido[1,2-a]pyrimidine-3-carbonitrile derivatives and establish a characterization framework for the new compounds.

- To prepare the methylthio-substituted aminopyrimidine intermediate using a guanidine/bis(methylthio)methylene malononitrile strategy.

- To investigate cyclocondensation of the intermediate with an appropriate methoxy-bearing 1,3-dicarbonyl precursor.
- To isolate and purify the proposed fused heterocyclic products.
- To characterize the products by IR, 1H NMR, 13C NMR, mass spectrometry and elemental analysis.
- To examine the synthetic utility of the C-2 methylthio group for subsequent nucleophilic substitution.
- To compare the spectroscopic features of the proposed dimethoxy series with the literature-reported dimethyl analogue.
- To establish a small analogue table that can be expanded after experimental optimization.

4. Proposed Molecular Design

The design combines four functional elements: (i) a fused pyrimidopyrimidine core, (ii) an imino functionality, (iii) a nitrile group, and (iv) a methylthio substituent. Two methoxy groups are introduced at positions 6 and 8. The methoxy groups are expected to increase electron donation through resonance and provide additional hydrogen-bond acceptor sites. Their presence may also influence chromatographic behavior, melting point and NMR chemical shifts.

A key design principle is modularity. The C-2 methylthio group is retained rather than immediately replaced because the reported analogue demonstrates that this position can be diversified with nucleophiles. Consequently, the proposed parent compound can function both as a target molecule and as a synthetic platform for a second-generation analogue library.

5. Proposed Synthetic Route

Step 1 — Formation of the aminopyrimidine intermediate:

Ethyl bis(methylthio)methylenemalononitrile is proposed to react with guanidine nitrate in the presence of potassium carbonate in DMF under heating. The literature precedent reports formation of 2-amino-1,6-dihydro-6-imino-4-(methylthio)pyrimidine-5-carbonitrile under these general conditions. Reaction monitoring should be performed by TLC and/or LC-MS.

Step 2 — Fused-ring construction:

The aminopyrimidine intermediate is proposed to undergo condensation with a suitably designed methoxy-substituted 1,3-dicarbonyl compound. The exact reagent should be selected according to the desired regiochemistry. A plausible mechanistic sequence involves nucleophilic addition/condensation, followed by intramolecular cyclization and aromatization or dehydrogenation as appropriate. Because substitution with methoxy groups changes the electronic character of the dicarbonyl partner, reaction time and acid/base conditions should be optimized experimentally.

Step 3 — Diversification:

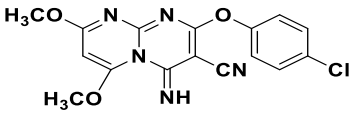
After isolation of the parent methylthio compound, nucleophilic substitution at C-2 may be investigated with selected amines, phenoxides or active methylene nucleophiles. The literature precedent indicates that the methylthio substituent can serve as a replaceable group in this class.

6. Proposed Reaction Mechanism

The first stage is interpreted as nucleophilic attack of guanidine-derived nitrogen on the activated bis(methylthio)methylene carbon, followed by displacement/condensation and ring closure to generate the substituted pyrimidine nucleus. The malononitrile-derived nitrile group remains available as a strongly electron-withdrawing substituent.

In the second stage, the amino functionality of the intermediate reacts with the carbonyl system of the dicarbonyl partner. Subsequent conjugate addition and/or condensation can generate an open-chain intermediate that is predisposed to intramolecular cyclization. The final ring closure produces the fused pyrimido[1,2-a]pyrimidine system. The exact sequence should be established or supported by control experiments and spectroscopic evidence rather than assumed solely from analogy.

The proposed mechanism is therefore a working mechanistic hypothesis. If the dimethoxy substrate gives an unexpected regioisomer, rearranged product or alternative cyclization product, the final manuscript should report the observed structure and revise the mechanistic scheme accordingly.



7. Materials and Methods

All solvents and reagents should be analytical or reagent grade. Reactions should be carried out using standard laboratory glassware under the conditions established by preliminary optimization. Thin-layer chromatography may be performed on silica-gel plates with UV visualization and suitable staining where required. Column chromatography, recrystallization or preparative TLC may be used for purification depending on product polarity.

7.1 Proposed preparation of aminopyrimidine intermediate

A literature-guided procedure begins by combining ethyl bis(methylthio)methylenemalononitrile and guanidine nitrate in DMF in the presence of potassium carbonate. The reaction is heated under reflux or an experimentally optimized temperature until consumption of the starting material is observed. After cooling, the mixture is worked up by an appropriate aqueous procedure and the crude product is purified. The reported study identifies the product as 2-amino-1,6-dihydro-6-imino-4-(methylthio)pyrimidine-5-carbonitrile.

The exact quantities, concentration, reaction time and work-up should be recorded in the laboratory notebook and inserted into the final manuscript. They are deliberately not fabricated here.

7.2 Proposed preparation of the dimethoxy fused product

The isolated aminopyrimidine intermediate should be reacted with a methoxy-bearing 1,3-dicarbonyl precursor selected to install the required 6,8-dimethoxy substitution pattern. The reaction can initially be screened under mild acidic or neutral conditions and then optimized using temperature, solvent and catalyst variables. Progress should be monitored by TLC and preferably LC-MS. Following completion, the reaction mixture should be neutralized where necessary, concentrated and purified. The isolated material should be dried to constant mass before characterization.

7.3 General characterization

- IR: record the spectrum from approximately 4000–400 cm−1 and identify NH, C≡N, C=N and C–O/C–O–C absorptions.
- 1H NMR: record in an appropriate deuterated solvent and assign methoxy, methylthio, ring and imino NH signals.
- 13C NMR: assign nitrile, imino/heteroaromatic carbons, methoxy carbon and methylthio carbon.
- Mass spectrometry: report the observed molecular ion/adduct and major fragment ions.
- Elemental analysis: compare calculated and experimental C, H, N and S values when available.
- Melting point: determine in duplicate or triplicate and report the observed range.
- Purity: report HPLC/LC-MS purity if available.

7.4 Safety considerations

DMF, nitrile-containing intermediates, sulfur-containing compounds and strong bases/acidic reagents should be handled according to institutional chemical-safety procedures. Appropriate gloves, eye protection and fume-hood use are required. Waste should be segregated according to local hazardous-waste rules. This manuscript does not replace an institutionally approved risk assessment.

8. Spectral Data and Structural Assignment

The closest published compound is the 6,8-dimethyl analogue. The 2025 report gives IR bands at approximately 3312 cm−1 for NH, 2235 cm−1 for C≡N and 1680 cm−1 for C=N; its reported 1H NMR includes a methylthio singlet near δ 3.25 and an imino NH signal near δ 8.35. The reported ESI-MS showed m/z 246 for the molecular ion. These values are literature data for the dimethyl compound and are not experimental data for the proposed dimethoxy target.

For the dimethoxy target, the following values should be treated as expected spectral regions only. Methoxy substitution is expected to introduce a 1H NMR singlet integrating for six protons, generally in the approximate δ 3.5–4.2 ppm region, and a 13C NMR methoxy carbon

commonly in the approximate δ 50–65 ppm region. The exact values depend strongly on solvent, concentration, tautomerism and the electronic environment of the fused ring.

Table 2. Spectral assignment framework

Technique	Expected feature	Approximate/assignment	Status
IR	N–H / imino	ca. 3200–3400 cm−1	Expected; verify experimentally
IR	C≡N	ca. 2200–2250 cm−1	Expected; verify experimentally
IR	C=N / conjugated ring	ca. 1600–1700 cm−1	Expected; verify experimentally
IR	Ar/heteroaryl C–O	ca. 1000–1300 cm−1	Expected; verify experimentally
1H NMR	OCH_3 × 2	ca. δ 3.5–4.2, s, 6H	Predicted range
1H NMR	SCH_3	ca. δ 2.3–3.5, s, 3H	Predicted range
1H NMR	=NH	broad/singlet; solvent dependent	Predicted; may shift
13C NMR	OCH3 × 2	ca. δ 50–65	Predicted range
13C NMR	C≡N	ca. δ 110–125	Predicted range
MS	Molecular ion/adduct	dependent on exact formula and ionization	Calculate and verify

8.1 Proposed molecular formula

If the proposed structure is represented as C13H15N5O2S, its approximate molecular weight is 305.36 g mol−1. This formula is a structure-based calculation and must be confirmed by the actual product structure and high-resolution mass spectrometry/elemental analysis. If the synthetic route produces a different oxidation or tautomeric state, the formula must be revised.

9. Results and Discussion Framework

Because no laboratory data were supplied with the request, this section is written as a results framework rather than as a claim of completed experimental work. The most important criteria for confirming formation of the dimethoxy pyrimido[1,2-a]pyrimidine core are the disappearance of starting-material signals, appearance of the expected nitrile and imino functionalities, observation of two methoxy groups in the 1H/13C NMR spectra, and a mass consistent with the proposed molecular formula.

A successful reaction should provide a single major product by TLC/LC-MS after optimization. The IR spectrum should retain a strong nitrile absorption and show an imino/NH band. In the 1H NMR spectrum, the two methoxy groups should normally appear as a combined six-proton singlet if they are chemically equivalent; non-equivalence could produce two separate signals. The methylthio group should appear as a three-proton singlet. The imino proton may be broad and solvent-dependent.

The 13C NMR spectrum should be particularly informative because it can distinguish the methoxy carbons from the methylthio carbon and provide evidence for the nitrile carbon. DEPT or HSQC experiments can assist in assigning protonated carbons, whereas HMBC correlations can help establish long-range connectivity in the fused heterocycle.

10. Comparative Analysis of the Dimethyl and Dimethoxy Designs

Parameter	6,8-Dimethyl analogue	6,8-Dimethoxy proposal	Expected consequence
Substituent	CH_3	OCH_3	Greater polarity/H-bond acceptance
Electronic effect	Weakly donating	Stronger resonance donation	May alter ring electron density
^{1}H NMR	Two methyl environments/signals	OCH_3 signal(s)	Diagnostic substitution change
^{13}C NMR	Aliphatic methyl carbons	OCH_3 carbons	Clear carbon-type change
IR	No strong ether C–O diagnostic	C–O bands expected	Additional fingerprint-region evidence
Molecular weight	Reported 246 for literature compound	Calculated ca. 305.36 for proposed formula	Higher mass
Diversification	C-2 methylthio available	C-2 methylthio retained	Same platform for substitution

11. Proposed Analogue Library

Once the parent dimethoxy compound is confirmed, a focused analogue library can be prepared by replacing the C-2 methylthio group with selected nucleophiles. The following table is a proposed research plan, not a report of completed syntheses.

Code	C-2 substituent	Purpose	Expected study
D1	Phenoxy	Neutral O-nucleophile	Electronic/lipophilicity comparison
D2	4-Methoxyphenoxy	Electron-rich O-nucleophile	Substituent effect
D3	Phenylamino	N-nucleophile	Hydrogen-bond donor/acceptor variation
D4	4-Methylphenylamino	Hydrophobic N-nucleophile	Lipophilicity effect
D5	4-Bromophenylamino	Halogenated N-nucleophile	Heavy-atom/substituent effect
D6	Pyrrolidin-1-yl	Aliphatic amine	Basicity and polarity variation
D7	Piperidin-1-yl	Aliphatic amine	Ring-size comparison
D8	Active-methylene substituent	C-nucleophile	Carbon–carbon diversification

12. Proposed Optimization Study

Optimization should be performed systematically rather than changing several variables simultaneously. A small matrix can evaluate solvent, temperature, catalyst and reaction time. The primary response variables should be conversion, isolated yield and chromatographic purity.

Entry	Solvent	Condition	Time	Observation
1	DMF	Heating; literature-guided	To be determined	Screening
2	Ethanol	Acid/base-assisted	To be determined	Screening
3	Acetonitrile	Mild heating	To be determined	Screening
4	DMF	Catalytic acid	To be determined	Optimization
5	DMF	Catalytic base	To be determined	Optimization
6	Solvent selected from above	Optimized temperature	To be determined	Final condition

13. Characterization and Confirmation Strategy

- TLC/LC-MS: confirm consumption of the aminopyrimidine intermediate and formation of a new major component.
- IR: confirm persistence of the nitrile and appearance of expected imino/ether features.
- 1H NMR: confirm the integrated proton count for two OCH3 groups and one SCH3 group.
- 13C NMR: identify the nitrile carbon and methoxy carbons; use HSQC/HMBC if assignments are ambiguous.
- HRMS: verify the exact mass of the proposed molecular formula.
- Elemental analysis: provide an independent bulk-composition check.
- HPLC: establish chemical purity where possible.
- If crystalline material is obtained, single-crystal X-ray diffraction would provide the strongest structural confirmation.

14. Anticipated Challenges

The principal synthetic challenge is control of regioselectivity during fused-ring formation. Methoxy substitution may alter the relative electrophilicity of carbonyl positions and therefore change cyclization efficiency. A second challenge is the possible presence of tautomeric forms involving the imino and amino functions. Such behavior can influence NMR chemical shifts and may complicate spectral assignment. A third challenge is purification because sulfur-containing heterocycles can show strong adsorption on silica and may require careful solvent selection.

If the target is not formed under the initial conditions, reasonable troubleshooting steps include changing the dicarbonyl precursor, adjusting acid/base strength, modifying temperature, varying

reaction concentration and testing alternative solvents. LC-MS should be used to determine whether failure arises from lack of reaction, decomposition or formation of an alternative cyclization product.

15. Summary Table of Proposed Results

Compound	Structural feature	IR evidence	NMR evidence	Status
Intermediate I	Aminopyrimidine + CN + SCH3	NH/CN/C=N	NH, SCH3 and heterocyclic signals	Literature-guided
Target T1	Dimethoxy fused ring	NH, CN, C=N, C–O	2×OCH3 + SCH3 + NH	Proposed
D1–D8	C-2 substituted derivatives	Core bands retained	New substituent signals	Future work

16. Conclusion

A literature-supported strategy has been developed for the proposed synthesis of 4-imino-6,8-dimethoxy-2-(methylthio)-4H-pyrimido[1,2-a]pyrimidine-3-carbonitrile derivatives. The approach is based on a closely related 2025 synthesis of the 6,8-dimethyl analogue, in which ethyl bis(methylthio)methylenemalononitrile and guanidine nitrate provide a methylthio-substituted aminopyrimidine intermediate followed by fused-ring construction. The proposed dimethoxy modification is chemically attractive because methoxy substituents can alter electronic properties and introduce additional hydrogen-bond acceptor functionality while retaining the reactive C-2 methylthio group.

The most important limitation of the present manuscript is that experimental data for the exact dimethoxy compounds were not supplied. Accordingly, no fabricated yield, melting point or spectrum has been presented as an observed result. Instead, the spectral section provides expected ranges and explicitly identifies the published dimethyl analogue as the experimental precedent. Once laboratory work is completed, the measured IR, NMR, HRMS, elemental analysis, melting point and chromatographic purity should replace the predicted values. This distinction is essential for a scientifically valid research paper.

Overall, the proposed scaffold represents a useful platform for synthetic diversification. Confirmation of the parent compound would enable preparation of a focused library of C-2-substituted derivatives and subsequent evaluation of their physicochemical, antimicrobial, antiproliferative or other biological properties using appropriate validated assays.

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