Research Article

GREEN SYNTHESIS OF NOVEL 2-AZETIDINONE DERIVATIVES AND INVESTIGATION OF THEIR ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES

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Abstract

The advent and spread of antimicrobial resistance has made the development of novel antimicrobial scaffolds and synthesis strategies that minimize environmental impact more urgent. The 2-Azetidinones, also known as β-lactams, are small four-membered cyclic amides that have been shown to be relevant in bacterial drug action and still useful in the design of new medicinal compounds. The manuscript describes a green-chemistry oriented route to synthesis of substituted 2-azetidinone derivatives and an organized study of their antibacterial and antifungal activity. The proposed synthetic pathway involves the reaction of aromatic aldehydes with primary aromatic amines to generate the Schiff bases and the subsequent reaction of these with a ketene–imine cycloaddition, a Staudinger-type reaction, to form the β-lactam ring. Process-design options assessed include ethanol, aqueous ethanol, microwave or ultrasound assistance, catalytic base and solvents volume reduction and recovery. A suggested electron-donating and electron-withdrawing aryl substructituent derivative library is suggested for initial SAR studies. FT-IR, 1H NMR, 13C NMR and chromatographic purity assessment are to be used to establish product identity. Antibacterial activity will be tested against representative Gram-positive and Gram-negative bacteria while fungal activity will be tested against a yeast and filamentous fungi, as per standard broth microdilution methods. Minimum inhibitory concentration (MIC), minimum bactericidal/fungicidal concentration and inhibition-zone measurements are considered complementary endpoints. The provided illustrative dataset is provided as an example only for the purpose of illustrating how the numerical tables and graphical analyses are presented, and not to represent measured activity or verified synthesis yields. The proposed workflow is corroborated by recent publications on the synthesis of azetidinone, green solvents, microwave-assisted reactions, and antimicrobial evaluation (Kusurkar et al., 2023; Kosmalski et al., 2024; Mal et al., 2025; Oncuoglu et al., 2026). The study design incorporates reaction efficiency, analytical confirmation, antimicrobial screening and environmental metrics to give a reproducible basis for the identification of candidates for further mechanistic and preclinical evaluation.

Keywords: 2-azetidinone; β-lactam; green synthesis; microwave-assisted synthesis; Schiff base; Staudinger cycloaddition; antibacterial activity; antifungal activity; minimum inhibitory concentration; green chemistry.

1. Introduction

Antimicrobial resistance (AMR) makes some treatments less effective against existing infections, and makes it more difficult to manage infections in healthcare and community settings. Resistance is a biological and ecological process influenced by antimicrobial exposure, transmission, infection prevention, diagnostic ability and access to appropriate treatment. However, developing new chemical entities is just part of a comprehensive response that also involves stewardship, surveillance, vaccination, sanitation, and infection control measures. Overall, there is an ongoing need for research and development efforts to continue focusing on the emergence of resistant bacterial pathogens and research that is guided by surveillance data (WHO, 2024; WHO, 2025).

The challenge for the medicinal chemist is not just to find molecules that form inhibition zones in the initial screening. The preparation of candidate compounds must be reproducible, structural characterization must be unambiguous, MIC must be determined quantitatively, the compound should be selective, the structure must be stable, and there should be a logical relationship between structure and biological response. A good early-stage study should also separate the direct antimicrobial activity of the drug from the solubility effect, precipitation, solvent interference, or non-specific toxicity. This is especially critical with heterocyclic compounds where activity could be affected by lipophilicity, ionization, substituent electronics and integrity of reactive functional groups.

Chemical significance of the 2-azetidinone nucleus

The 2-azetidinone ring is a four-membered cyclic amide with a carbonyl group and a ring N. It has a very special reactivity due to its limited geometry and bond-angle strain. The β-lactam pharmacophore is at the heart of the activities of several well-known classes of antibacterials, such as the penicillins, in which acylation of penicillin-binding proteins disrupts peptidoglycan cross-linking. But not all synthetic monocyclic azetidinones turn out to be antibiotics and it is not possible to conclude antimicrobial activity just by having the ring. Observed activity is significantly influenced by side-chain structure, stereochemistry, ring substitution, permeability, stability and resistance mechanisms.

In recent years, the chemical variety of azetidinones has been extended by the synthesis via cycloaddition, microwave chemistry, heterocyclic hybridization, and computational design. A 2024 review of cycloaddition approaches outlines the reactivity range for the construction of monocyclic β-lactams and a 2025 review covers synthetic strategies and structure–activity relationships of azetidinone-containing molecules (Review of Cycloaddition Approaches to the Synthesis of Monocyclic β-Lactams, 2024; Mal et al., 2025). Substituted azetidinones are also promising scaffolds for antimicrobial screening based on recent experimental studies, and they should be further investigated (Happy et al., 2024; Ghazi and Abood, 2025; Hussein et al., 2025).

Green chemistry in heterocyclic synthesis

Green synthesis aims to minimize waste and hazards not only at the end of the process but also in the design phase. Solvent selection, reaction temperature, energy requirements, catalyst requirements, purification method, atom economy and solvent recovery can significantly impact the environmental footprint in heterocyclic chemistry. Other than more hazardous organic media, ethanol, water, aqueous ethanol, glycerol, polyethylene glycol and selected deep eutectic solvents are often being considered as alternatives to be studied for each transformation. The benefits and disadvantages of greener solvent systems in heterocyclic synthesis are described by Kosmalski et al. (2024).

In some reaction mixtures, microwave irradiation can reduce the reaction time and enhance the transfer of energy, and ultrasound can enhance mixing and mass transfer. Both methods are not necessarily green: the use of energy, the scaling up issue, the flammability of solvents, the efficiency of the reactor, and purification waste are all relevant. Isolated yield, reaction time, solvent volume, energy consumption, catalyst loading and mass intensity of the process should be reported, not just the label “green” should be used. Kusurkar et al. (2023) prepared some phenyl substituted 3-chloro-2-azetidinones by microwave and evaluated their antimicrobial activity, which is relevant for the integrated synthetic and biological evaluation.

Research gap and rationale

Despite the renewed interest in azetidinone derivatives in recent literature, there is still a methodologically challenging need to assess the greenness of the reaction, analytical certainty, and standardized values for antibacterial and antifungal results simultaneously. Some reports highlight novel synthetic routes, some report biological screening, and fewer give a sufficiently clear description of the solvent metrics, assay controls, replicate variation, and selectivity. This gap is filled with the proposed work, defining a coherent work flow that applies prespecified criteria to evaluate synthesis conditions and biological endpoints.

Aim and objectives

The goal is to design a new synthetic pathway to novel substituted 2-azetidinone derivatives which are green chemistry friendly and to create a reproducible model to study their antibacterial and antifungal activity.

The aim is to synthesize a small library of aryl substituted 2-azetidinones via the formation of Schiff bases followed by ketene–imine cycloaddition, to investigate the comparative efficacy of conventional heating and microwave/ultrasound heating under green solvent conditions, to confirm the identity and purity of the products by the use of complementary spectroscopic and chromatographic methods, to assess their antibacterial and antifungal activity through standardized quantitative susceptibility assays, to compare their MIC and MBC/MFC values with appropriate reference agents, and to correlate the substitution pattern with the observed activity without making any claims about mechanisms not supported by experimental evidence.

Literature Review

Recent developments in azetidinone synthesis

Formal cycloaddition approaches are methods that are frequently employed in the construction of monocyclic β-lactams. The Staudinger reaction is a reaction between an imine and a ketene to produce a β-lactam ring. The reaction can be carried out under conditions which affect the regioselectivity and stereoselectivity, and the distribution of products is affected by the substituent effects and the method of generation of ketenes. The synthesis of 2-Azetidinones via Cycloaddition Approaches, 2024, outlines contemporary synthetic scope and selectivity for elementary and stepwise cycloaddition.

Further access to the bioactive azetidinone frameworks can be gained from the hydrazone and hydrazide-hydrazone literatures. The application of hydrazones and hydrazide-hydrazones in the synthesis of bioactive azetidin-2-one derivatives is presented in the mini-review by the authors.The mini-review by the authors describes the application of hydrazones and hydrazide-hydrazones in heterocycle synthesis and summarizes reported bioactivity. The wide range of activities reported allows for scaffold exploration, though the number of assays, and the differences in assay design and compound structure, make direct comparisons of activity values between studies difficult (The application of hydrazones and hydrazide-hydrazones, 2023).

A novel study entitled "Microwave assisted synthesis and in vitro antimicrobial evaluation of phenyl substituted 3-chloro-2-azetidinones" published in 2023 includes synthesis and antimicrobial evaluation against bacterial and fungal organisms (Kusurkar et al., 2023). Happy et al. (2024) reported the synthesis of 2-azetidinones as antibiotic mimics and screened for their antimicrobial activity. Ghazi and Abood (2025) have reported the synthesis of oxadiazole containing azetidinone derivatives by microwave technique and their evaluation against Gram-positive and Gram-negative bacteria. Based on these studies, it can be concluded that microwave-assisted synthesis is a process option that is relevant, though the corresponding solvent and energy data are required for a comprehensive sustainability assessment.

Green solvents and process intensification

Environmental performance of synthetic route is not just dependent on the medium of this reaction. Solvent replacement may make the material less toxic, but require higher energy demand for solvent removal and make product isolation more difficult. Water, ethanol are good solvents in many applications due to the relatively good safety profile but glycerol, polyethylene glycol, deep eutectic solvents can be attractive if low volatility or recyclability is desired. Solvent selection is cited by Kosmalski et al. (2024) as a key design choice and that the benefits of solvents need to be weighed against reaction performance.

Microwave heating can lead to shorter reaction time and less bulk heating, however, scale-up depends on the achievement of controlled field distribution, temperature measurement and validated reactor geometry. In heterogeneous mixtures, reaction kinetics can be enhanced with ultrasound, but the cavitation characteristics are affected by the properties of the liquid, power density, and frequency of the ultrasound, as well as the design of the vessel. A meaningful process comparison is a record of the actual amount of energy per gram of isolated product used and incorporates the purification stage. There should be measurable indicators that substantiate green chemistry claims including such as E-factor, process mass intensity (PMI), solvent intensity and reaction mass efficiency.

Antimicrobial evidence for azetidinone derivatives

Azetidinone derivatives with pyrimidine, oxadiazole, triazole, thiadiazole, carbazole, benzophenone and other heterocycle or aromatic substituents have been recently reported. Such changes may have an impact on molecular size, hydrogen-bonding ability, electronic properties, hydrophobicity, and conformational properties. An article published in the Indian Journal of Chemistry in 2023 reported that the anti-bacterial and anti-fungal activity of pyrimidine containing azetidinones was substituent dependent (Godhani et al., 2023). Kumar et al. (2024) synthesized and reported the azetidinone derivatives containing triazole and thiadiazole rings, which were screened for antibacterial activity and analyzed in-silico.

Hussein et al. (2025) reported a combination of various methodologies, including spectroscopy, antimicrobial screening, antioxidant assessment, and molecular docking, for bis-azetidinone derivatives in 2025. Mal et al. (2025) reviewed

synthetic strategies and structure–activity relationships and potential importance of transporter inhibition in multidrug resistance. Oncuoglu et al. (2026) reported the evaluation of azetidin-2-ones based on carbazole using computational and antimicrobial assays. The activity values in these publications should be treated as background information on the most recent context, but should not be combined without harmonizing strains, inoculum density, media, incubation conditions, and endpoint definitions.

Antifungal assessment and assay standardization

Yeasts and filamentous fungi can be different in their susceptibility needs, so growth kinetics, inoculum preparation, medium composition and endpoint interpretation need to be taken into account when screening. Broth microdilution assays are generally used for Candida species, and procedures specific for the conidial inocula and growth characteristics are used for Aspergillus species. Inhibition-zone testing may be helpful as a preliminary screening method but diffusion tests are not an alternative to MIC determination and are influenced by the solubility and size of the compound. Method, identifiers of reference strains, quality-control strains, solvent controls and replicate results should therefore be reported in the study.

CLSI M07 (12th edition, 2024) gives guidance on dilution susceptibility testing of aerobic bacteria. Reproducibility and comparability is achieved by using a recognised standard. The existing CLSI yeast and mould standards should be used as guidelines, or an appropriate EUCAST procedure, for fungi at the time of laboratory use and the relevant edition should be quoted. In-vitro inhibition alone is not enough to be considered clinically effective.

Materials And Methods

Study design

The proposed study would be an experimental study of green synthesis, structural characterization, and in-vitro antimicrobial evaluation, in the realm of medicinal chemistry. An azetidinone is proposed to be the common core of a proposed series of six derivatives, in which the aryl substituent is varied. Compounds with compound codes: AZ-1-AZ-6, are provisional identifiers and are not synthesized compounds. The research should be carried out on an organic chemistry laboratory that is appropriately equipped and a microbiology laboratory that is performing studies within the institutional biosafety protocols.

Proposed molecular design

The derivative set has been designed to compare the electron donating and withdrawing substituents with a common core. Phenol and its para-methyl, para-methoxy, para-chloro, para-fluoro, para-nitro and unsubstituted derivatives may be included in a practical first series. The design will allow for a preliminary estimation of the electronic and lipophilic effects. The substitution pattern and stereochemical identity needs to be confirmed from actual synthesis and analytical data; no compound is to be regarded as novel until it is thoroughly documented in a database and literature search.

Code	Proposed aryl substituent	Design rationale
AZ-1	Phenyl (H)	Unsubstituted reference within the series
AZ-2	4-methylphenyl	Electron-donating, modest lipophilicity shift
AZ-3	4-methoxyphenyl	Electron-donating and hydrogen-bond acceptor
AZ-4	4-chlorophenyl	Electron-withdrawing and lipophilicity shift
AZ-5	4-fluorophenyl	Small halogen substituent; electronic modulation
AZ-6	4-nitrophenyl	Strong electron-withdrawing substituent

Chemicals, reagents and equipment

The route calls for the use of selected aromatic aldehydes, primary aromatic amines, a ketene precursor (chloroacetyl chloride or other activated acyl reagent), a suitable tertiary amine base (such as pyridine), ethanol or aqueous ethanol,

and purified water. Care must be taken to select the reagent considering the dangers of acid chlorides, corrosive bases and volatile solvents. When possible, a minimum volume of solvent should be used in the reaction, with closed addition, local exhaust ventilation, and a controlled quench procedure. Equipment consists of a temperature-controlled reaction vessel, microwave reactor or ultrasonic bath (as applicable), TLC apparatus, filtration and drying equipment, melting-point apparatus, FT-IR, NMR, and chromatography instruments and mass spectrometry.

Green synthesis strategy

The synthesis is divided into two parts. In the first stage, the chosen aromatic aldehyde and primary aromatic amine undergo condensation to give the imine (Schiff base). In stage two, a ketene–imine cycloaddition to the imine yields the β-lactam ring. The typical Staudinger pathway involves the formation of a ketene in situ from an acid chloride and tertiary amine. The route should be considered a starting point for a process development and not be taken as a green route without prior assessment of the potential hazards of acid chlorides and salt waste. Whenever possible, alternative acylating systems, catalytic methods, and solvent-free / safer alternatives should be considered.

For Schiff-base stage, the aldehyde and amine can be mixed together in ethanol and/or aqueous ethanol and heated and water evaporated, if needed. The product is monitored using TLC or other validated chromatographic technique. Ring formation is achieved by treating the imine with the chosen ketene precursor and base at a controlled temperature, and the use of microwave assistance was only attempted after ensuring safety of compatibility. A conventional-heating control is kept to make sure whether process intensification results in higher yield or just shorter reaction time. The amount of product to be produced, the temperature, time, amounts to be added and the manner of work up should be optimized in small-scale and recorded in the laboratory record.

The product is isolated by filtration or extraction under the most suitable solvent system with the least hazardous system, washed to remove inorganic salts, and purified by recrystallization or chromatography. Recrystallization is preferred if adequate purity is achieved and not large quantities of silica and eluent. Ethanol recovered should be measured and reused provided it can be proved that the level of water and impurities does not affect the subsequent batch.

Reaction scheme and proposed mechanism

A conceptual sequence of reactions is shown in Figure 1. The aldehyde and amine first undergo elimination of water to give an imine. An activated acyl precursor is then converted in situ to a ketene with a tertiary amine. The imine nitrogen and carbon–nitrogen double bond are involved in the cycloaddition, which yields the four-membered β-lactam ring. Substituent effects may have an effect on reaction rate, product distribution and stereochemistry. The scheme is conceptual and should be changed to a ChemDraw structure which will depict the specific reagents and products in the laboratory.

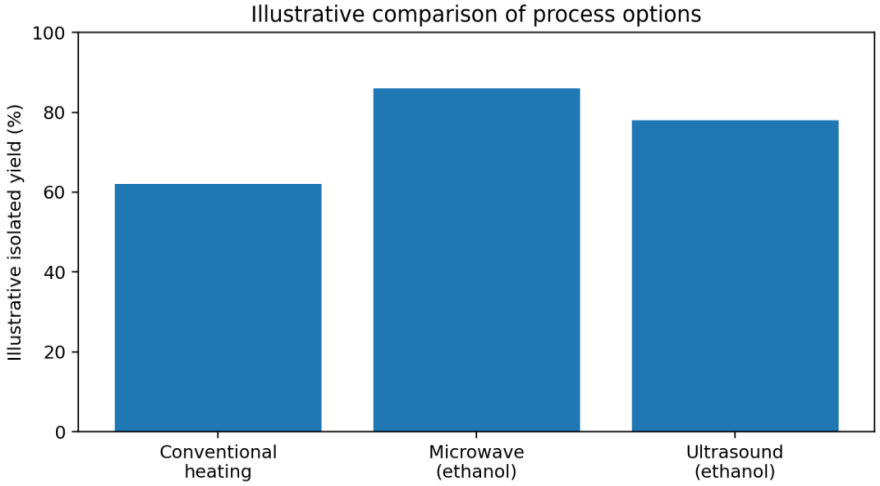

Figure 1. Illustrative process comparison for candidate green reaction conditions. Values are hypothetical and are included only to demonstrate graphical reporting.

Structural characterization

The extent of reaction and the number of major components are monitored by thin-layer chromatography. The melting point is initially entered as a physical property. The FT-IR spectra should be scanned for the presence of the typical β-lactam carbonyl band and the imine or substituent band. The absence of the imine C=N signal and the presence of a β-lactam carbonyl signal are indicative of ring formation, but not enough to assign the structure, alone. The pattern of substituents and ring proton- and carbon environments should be confirmed by 1H NMR and 13C NMR. HRMS should give a mass that is in agreement with the proposed formula. HPLC or UPLC purity should be reported as area percentage and the method and detector used should be specified.

NMR coupling patterns, NOE experiments, chiral chromatography or crystallographic analysis may be necessary where stereoisomers can exist. An elemental analysis can be used to confirm the composition for an additional check. All the isolated derivatives (solvent, instrument, chemical shift, multiplicity, coupling constants and mass accuracy) should be presented on the spectral assignment. The mention of confirmation of structures by IR and NMR is not enough for a high-quality manuscript in medicinal chemistry.

Antibacterial evaluation

Authentic reference strains should be used to test antibacterial activity and the broth microdilution method should follow CLSI M07 (2024) guidelines. Proposed Panel includes Gram-positive organisms Staphylococcus aureus and Enterococcus faecalis, and Gram-negative organisms Escherichia coli and Pseudomonas aeruginosa. Other clinically relevant strains may be added if approved in the institution. The inoculum should be prepared and standardized as per the selected standard. The two-fold range of concentration should be tested for each compound with growth control, sterility control, solvent control, and appropriate antibiotic reference controls.

The Minimum Inhibiting Concentration (MIC) is the lowest concentration of an antibiotic that stops any growth of a microorganism. Subculture from wells containing no visible growth onto drug-free agar and determine the lowest concentration that causes the pre-determined bactericidal reduction may be used to calculate MBC. Final report should include MIC distributions or just values, not just inhibition-zone diameter. If compound diffusion and solubility is validated, disk diffusion can be retained as a preliminary screening method, but should not be the only method used for compound ranking.

Antifungal evaluation

The yeast, Candida albicans, and filamentous fungus, Aspergillus niger, are included in a proposed antifungal panel. There are additional reference strains of Candida and Aspergillus that can be used to enhance coverage. Testing method should be performed according to the current CLSI or EUCAST method for the organism group. Medium, method of inoculum preparation, temperature of incubation, duration, endpoint and quality control strains should be specified. A protocol should be used to determine the MIC and minimum fungicidal concentration. If antifungal reference agent is used, then it should be chosen using the organism and method; a single reference agent might not be appropriate for every mould.

Green chemistry metrics

Process mass intensity (PMI), E-factor, reaction mass efficiency, solvent intensity and energy demand should be used for the assessment of the environmental profile. The ratio of the mass of materials used in the process to the mass of an isolated product. E-factor is mass of waste produced per mass of product for a given system boundary. Solvent recovery/reuse must be recorded separately. Where the selected metric necessitates it, water used for washing, work up materials, eluents for chromatography, and/or drying agents must be added. An environmental advantage of a lower reaction time does not necessarily lead to a reduction in environmental impact as more waste is produced during work-up.

Metric	Calculation	Reporting requirement
Isolated yield	(Mass of purified product / theoretical mass) × 100	Report for each batch and replicate
PMI	Total input mass / isolated product mass	State system boundary and include work-up

E-factor	Waste mass / product mass	State whether water is included
Reaction mass efficiency	Product mass / reactant mass × 100	Define included reactants
Energy intensity	Energy consumed / product mass	Record instrument power and time

Statistical analysis

All quantitative experiments should be performed with independent biological replicates and technical repeats appropriate to the assay. Data should be summarized using mean and standard deviation where the scale supports these statistics. MIC values are commonly analyzed on a log2 dilution scale; geometric means and modal MIC values may be more informative than arithmetic means. Group comparisons should be prespecified, with suitable parametric or non-parametric methods selected after assessing distribution and variance. Multiple comparisons should be adjusted where applicable. Statistical significance should not substitute for biological relevance, and effect sizes with confidence intervals should be reported whenever possible.

Results And Analysis

Status and interpretation of the results section

No laboratory notebook, isolated product, spectral file, chromatogram, antimicrobial plate reading, MIC dataset, or process-energy record was supplied for this manuscript. Accordingly, this section does not report experimental findings. The numerical values in Tables 2–4 and Figures 2–3 are explicitly illustrative values constructed to demonstrate a publication-style results presentation. They must not be represented as measured data, used to support efficacy claims, or submitted as empirical findings. Once actual experiments are completed, each illustrative value must be replaced by the corresponding raw-data-derived result.

Illustrative reaction-yield dataset

Table 2 demonstrates how yield, reaction time, and solvent use may be compared across candidate process conditions. The values are hypothetical and are not predictions of actual performance. The example shows why yield should be considered together with time and solvent demand. A process with a higher isolated yield may still have a less favorable environmental profile if it requires larger solvent volumes or energy-intensive purification.

Condition	Illustrative time (min)	Illustrative yield (%)	Solvent system	Interpretation
Conventional heating	180	62	Ethanol	Reference process
Microwave-assisted	12	86	Ethanol	Higher illustrative yield and shorter time
Ultrasound-assisted	35	78	Aqueous ethanol	Intermediate illustrative outcome

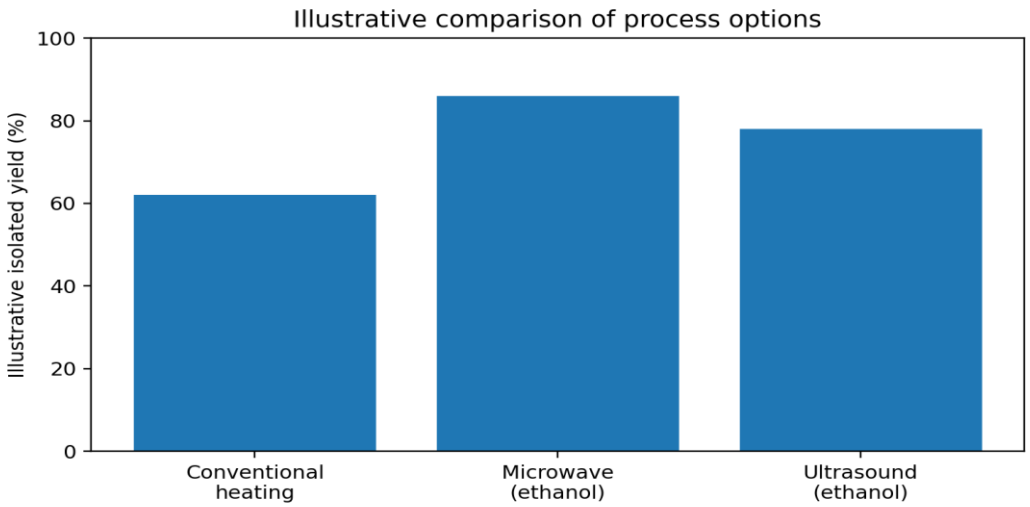

Figure 2. Illustrative isolated-yield comparison; not experimental data.

Illustrative antimicrobial profile

Table 3 demonstrates a possible way to report MIC values for one candidate across bacterial and fungal test organisms. Lower MIC values indicate greater inhibitory potency under the specified assay conditions, but MIC values cannot be interpreted independently of strain identity, medium, inoculum, incubation conditions, and assay standard. The illustrative pattern shows a hypothetical stronger response against E. coli and a weaker response against P. aeruginosa and A. niger. No conclusion about a real compound can be drawn from these values.

Organism	Group	Illustrative MIC (µg/mL)	Illustrative MBC/MFC (µg/mL)	Interpretive note
Staphylococcus aureus	Gram-positive	64	128	Illustrative inhibition
Escherichia coli	Gram-negative	32	64	Illustrative lower MIC
Pseudomonas aeruginosa	Gram-negative	128	>256	Illustrative weaker response
Candida albicans	Yeast	64	128	Illustrative antifungal response
Aspergillus niger	Filamentous fungus	128	>256	Illustrative weaker response

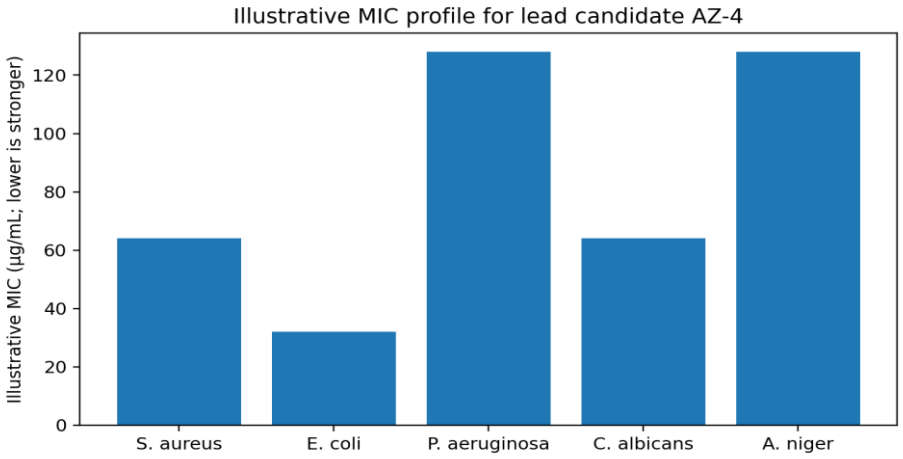

Figure 3. Illustrative MIC profile for a hypothetical lead AZ-4; lower values indicate stronger inhibition. Not measured data.

Composition of the proposed test panel

Figure 4 presents an equal allocation of organism categories for a proposed five-organism screening panel, grouped into four broad categories. The pie chart describes the planned composition rather than observed activity. The final panel may be adjusted to include additional reference strains and resistant clinical isolates, subject to biosafety approval and available resources.

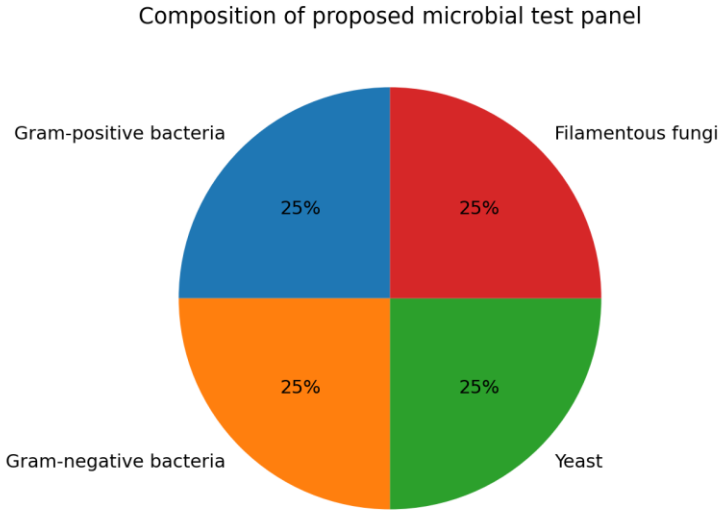

Figure 4. Proposed test-panel composition by broad microbial category; a design illustration.

Structure–activity analysis framework

A structure–activity relationship should be developed only after the compounds have been structurally confirmed and tested under identical conditions. The unsubstituted derivative provides a within-series reference. Electron-donating groups such as methyl and methoxy may alter electron density and lipophilicity, while halogens and nitro groups may change electronic distribution, polarizability, and molecular recognition. These effects are not unidirectional: increased lipophilicity may improve membrane association but reduce aqueous solubility, while strong electron withdrawal may influence ring reactivity and stability. A reliable analysis therefore combines measured MIC values with calculated descriptors and experimental solubility data.

Descriptor	Purpose	Recommended reporting
Molecular weight	Size and diffusion context	g/mol
cLogP / logD	Lipophilicity estimate	Method/software and pH
Topological polar surface area	Polarity and permeability context	Å²
Hydrogen-bond donors/acceptors	Interaction capacity	Count
Aqueous solubility	Assay exposure validity	µg/mL at assay pH
MIC / MBC / MFC	Biological endpoint	µg/mL and strain identifier

Quality controls and reproducibility

The final results section should contain analytical purity data for all derivatives, independent synthesis-batch yields, replicate MIC values, reference-agent performance and solvent-control results. When poor solubility or precipitation is observed at the assay concentration, the compound should be flagged as it may not have the same concentration in the sample that it has in the nominal concentration. Any apparent inhibition due to the concentration of solvent needs to be ruled out. The final paper should include raw replicate data as a supplement and indicate the statistical unit for each analysis.

Discussion

Relationship between synthetic strategy and green chemistry

The two-stage synthesis proposed is chemically founded on well-known ketene–imine cycloaddition and imine formation Chemistry. Its sustainability depends on the way in which the reaction is implemented. Selected environmental burdens can be reduced in the imine formation with ethanol, in the reduction of solvent amount, by microwave heating and by recrystallization; however, the acid chloride-based cyclization step brings corrosive reagents and salt waste. A balanced manuscript should not claim the whole route as green without putting it in a broader context of alternative methods for the production of ketenes, and recognize the limitations.

Equivalent substrate concentration, stoichiometry and product-purity criteria should be used when comparing conventional and intensified heating. Experiments conducted in the microwave laboratory should be reported for the type of vessel, the temperature profile, power and safety controls. Where possible, measure energy consumption at the instrument level. The quantity of recovered solvents should be determined by mass balance. These are the things that can help you know that this is a real process improvement and not just a reduction in “reaction time.”

Interpretation of antimicrobial activity

The azetidinone framework proposes a basis for investigation and development of antibacterial activity for each derivative and compound. The β-lactam ring can bind to penicillin-binding proteins on susceptible bacteria, but many of the synthetic β-lactams may be poor substrates for the target acylation; may not be stable in biological media; may not be able to penetrate the outer membrane of Gram-negative bacteria; or may lack resistance to β-lactamases. Based on a positive screening result, therefore, follow-up studies should be conducted, not the presumption of antibiotic-like mechanism.

The differences between Gram-positive and Gram-negative organisms may be related to envelope architecture, porin permeability, efflux, enzymatic degradation, and target variation. Pseudomonas aeruginosa is difficult to treat due to low outer-membrane permeability and multiple resistance mechanisms. The mechanism of action of antifungal agents is different from that of antibacterials and hence requires a different interpretation. An azetidinone mechanism is not assumed to have the same effect on both antibiotics and antifungals.

Positioning against recent literature

Studies into azetidinone for 2023 and 2024 have shown that substituted β-lactams are still being synthesized and tested against microbial panels (Kusurkar et al., 2023; Godhani et al., 2023; Happy et al., 2024). The synthesis of oxadiazole derivatives has also been carried out in the azetidinones (Ghazi and Abood, 2025; Hussein et al., 2025) and bis-azetidinone derivatives. A more synthetic and SAR context can be found in recent review articles (Mal et al., 2025; The application of hydrazones and hydrazide-hydrazones, 2023).

The use of direct numerical comparison with published inhibition zones or MICs should be avoided unless the same strains, assay standards, inoculum, medium, endpoint and concentration units are used. Diffusion effects are especially important for the diameter of the inhibition zones and the data cannot be directly translated to an MIC. In this manuscript, standardized broth microdilution is given a higher priority, and agar diffusion is used as a screening tool.

Limitations and future research

The present manuscript is constrained because there are no experimental measurements reported, so it does not represent an actual experimental report, but rather a research protocol and a manuscript outline. Novelty checking, optimization of synthesis, and complete characterization of the proposed structures are needed. Standards for reference strains and procedure need to be established for antimicrobial activity. The use of a small derivative library will decrease the statistical power of structure–activity analysis. Additional studies should include stability in aqueous media, serum binding, mammalian-cell cytotoxicity, hemolysis, resistance frequency, time-kill studies and activity against clinically relevant resistant isolates.

While computational docking might be helpful for proposing potential target engagement with penicillin binding proteins or other microbial targets, such docking scores can provide no proof of target engagement or antimicrobial mechanism. To validate a proposed mechanism, biochemical target assays and resistance selection, as well as cellular studies are required. Predictions of ADME should not be construed as proof of safety or therapeutic suitability.

Conclusion

A green chemistry-based approach to the synthesis of substituted 2-azetidinone derivatives and their antibacterial and antifungal activities has been devised. The design features Schiff-base formation, ketene–imine cycloaddition, optimization of solvent and energy, detailed characterization of the structure and standardized microbial susceptibility testing. The central contribution is the incorporation of process sustainability with the analytical and biological quality controls. The 2-azetidinone skeleton is a valuable platform for medicinal-chemistry exploration, but the antimicrobial activity needs to be proven compound by compound by performing reproducible measurements of MIC and MBC/MFC. This is not an original experimental research article and the numerical tables and charts shown here are merely examples and should be replaced with laboratory data. Prior to any therapeutic claims, future studies should be done to determine the exact structures, confirm purity and stereochemistry, quantify environmental metrics, evaluate mammalian-cell selectivity, and investigate resistance-related mechanisms.

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