



Green Synthesis of Benzimidazole Derivatives and Their Antimicrobial Evaluation: A Review

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ABSTRACT :

Antimicrobial resistance and the environmental cost of conventional heterocyclic synthesis are two pressing problems in medicinal chemistry. Benzimidazole is a privileged scaffold with documented antibacterial and antifungal activity, but its classical syntheses often depend on strong acids, high temperatures, long reaction times, and organic solvents. This review summarises green synthetic approaches to benzimidazole derivatives, including water and solvent-free media, microwave and ultrasound activation, mechanochemistry, deep eutectic solvents and ionic liquids, and reusable heterogeneous and nanocatalysts, and relates them to reported antimicrobial evaluation. Structure–activity trends for antibacterial and antifungal activity and standardised methods for susceptibility testing are discussed. Current limitations include inconsistent reporting of green metrics, variation in assay methods between studies, and limited mechanistic and toxicity data. A stepwise strategy linking green synthesis, characterisation, antimicrobial screening, and early safety assessment is proposed.

Keywords: benzimidazole; green synthesis; antimicrobial activity; antibacterial; antifungal; microwave; deep eutectic solvents; nanocatalyst

1. Introduction: Antimicrobial Resistance and the Case for Greener Synthesis

Antimicrobial resistance (AMR) is a major threat to global health. A systematic analysis estimated that bacterial AMR was associated with about 4.95 million deaths in 2019, of which about 1.27 million were directly attributable to resistance [1]. Fungal infections add to this burden, with an estimated 1.5 million or more deaths each year [2]. Resistance in fungi arises through mechanisms such as efflux, target alteration, and changes in the ergosterol pathway [3]. New chemical scaffolds, and more sustainable ways to make them, are therefore both needed.

Benzimidazole, formed by fusing benzene and imidazole, is a privileged scaffold found in marketed anthelmintic, antiulcer, antihypertensive, and anticancer drugs [4,5]. Its antimicrobial potential has been reviewed repeatedly [6,7].

Benzimidazoles are classically prepared by the Phillips condensation of *o*-phenylenediamine with a carboxylic acid under strong acid [8], or by condensation with aldehydes followed by oxidative cyclisation. These routes can need harsh conditions, hazardous solvents, and extensive purification [9,10]. Green chemistry offers a framework for improving them, built on principles such as waste prevention, safer solvents, energy efficiency, and catalysis [11,12].

This review concentrates on green synthetic methods for benzimidazole derivatives and on the antimicrobial evaluation of the compounds obtained. Broader accounts of benzimidazole synthesis are available elsewhere [10,13].

2. Benzimidazole as an Antimicrobial Scaffold

2.1 Structural features

The benzimidazole ring carries an N–H group that can donate a hydrogen bond and an sp²-hybridised nitrogen that can accept one, and its planar aromatic system supports π – π stacking. Because the bicyclic core resembles purine, derivatives can interact with nucleotide-binding biomolecules [4].

2.2 Sites of substitution

Positions N-1, C-2, and C-5/C-6 are the main sites varied to tune activity, and structure–activity relationship (SAR) studies have been reviewed for several biological activities [5,14].

2.3 Antibacterial and antifungal targets

In fungi, ergosterol biosynthesis is a principal target of current antifungal drugs, and lanosterol 14 α -demethylase is a key enzyme in this pathway [15].

Crystal structures of this enzyme from *Candida albicans* and *Candida glabrata* provide tools for antifungal design [16]. For bacteria, the targets proposed for benzimidazoles differ between series and are often not confirmed experimentally [6,7].

3. Green Chemistry Principles Applied to Benzimidazole Synthesis

The twelve principles of green chemistry cover waste prevention, atom economy, safer solvents, energy efficiency, and catalysis [11,12]. The E factor, defined as the mass of waste per mass of product, is a widely used metric [17], and solvents are often the largest contributor to process waste, which makes solvent choice central to greener design [18].

For benzimidazole routes, greenness is better judged by E factor, reaction time, energy input, solvent, and catalyst reuse than by yield alone. Reporting these measures alongside yield would make claims of greenness comparable between studies.

4. Green Synthetic Strategies

4.1 Water as reaction medium

Water is non-toxic, inexpensive, and non-flammable, and organic reactions in water have been reviewed in detail [19]. Aqueous routes to benzimidazoles are described in broader synthetic reviews [10,13]. Poor solubility of organic substrates is the main limitation.

4.2 Solvent-free and mechanochemical methods

Solvent-free reactions remove the largest source of waste in many syntheses [20]. Mechanochemistry, in which grinding or milling drives the reaction, offers a cleaner alternative to solution chemistry [21]. Their suitability for each benzimidazole route should be assessed individually, particularly for scale-up and heat control.

4.3 Microwave-assisted synthesis

Controlled microwave heating can shorten reaction times substantially compared with conventional heating [22]. A comparison of conventional and microwave-assisted synthesis of benzimidazole and its 2-substituted derivatives reported shorter reaction times and improved yields under microwave conditions [23].

4.4 Ultrasound-assisted synthesis

Ultrasound is another energy-efficient activation method, in which cavitation can accelerate reactions under mild bulk conditions [24]. Its application to benzimidazoles is covered in broader synthesis reviews [13].

4.5 Deep eutectic solvents and ionic liquids

Deep eutectic solvents (DES), mixtures of hydrogen-bond donors and acceptors with low melting points, are regarded as greener media [25], and room-temperature ionic liquids have long been used as solvents for synthesis and catalysis [26]. In one study, a DES served as both reaction medium and reagent for the selective synthesis of 2-substituted or 1,2-disubstituted benzimidazoles from o-phenylenediamine and aldehydes, with advantages in yield and work-up [27].

4.6 Heterogeneous and nanocatalysts

Reusable heterogeneous catalysts reduce waste and simplify product isolation. A reusable nano-zirconia catalyst has been used for the synthesis of benzimidazoles, and the products were then evaluated against Gram-positive bacteria, Gram-negative bacteria, and fungi, with satisfactory activity reported [28]. Milder catalytic methods for 2-arylbenzimidazoles have also been described [29]. Catalyst cost, preparation, leaching, and removal must be considered for scale-up.

Table 1. Green strategies for benzimidazole synthesis.

Strategy	Key features	Advantages	Limitations
Water [19]	Aqueous reaction medium	Safe, inexpensive	Poor solubility of organic substrates
Solvent-free / mechanochemical [20,21]	Neat reaction, grinding or milling	No bulk solvent, simple work-up	Scale-up and heat control
Microwave [22,23]	Dielectric heating	Short reaction times, improved yields reported	Specialised equipment, scale-up

Strategy	Key features	Advantages	Limitations
Ultrasound [24]	Acoustic cavitation	Mild conditions, energy efficient	Reactor reproducibility
DES / ionic liquids [25,26,27]	Alternative solvents, can act as reagent	Recyclable, can improve selectivity	Viscosity, product isolation, toxicity data for some ionic liquids
Reusable / nanocatalysts [28,29]	Heterogeneous catalysis	Catalyst recovery, mild conditions	Catalyst preparation cost, leaching

5. Antimicrobial Evaluation of Green-Synthesised Benzimidazoles

5.1 Does the synthetic route affect activity?

A green route changes how a compound is made, not its structure, so antimicrobial activity depends on structure and purity rather than on the synthetic method. The benefit of green synthesis lies in sustainability and, potentially, in cleaner products with fewer residual acid or metal impurities that could confound biological assays.

5.2 Antibacterial activity

Benzimidazoles have been investigated extensively as antibacterial agents [6], and a review covering 2018 to 2024 analyses their SAR [7]. Lipophilic electron-withdrawing substituents are reported to enhance antibacterial activity in several series [6,7]. Benzimidazoles prepared through the nano-zirconia route showed activity against Gram-positive and Gram-negative bacteria [28]. Gram-negative organisms are often less susceptible because of their outer membrane and efflux systems, so activity should be reported separately for each group.

5.3 Antifungal activity

Bis-benzimidazoles have shown minimum inhibitory concentration (MIC) values in the low $\mu\text{g/mL}$ range against several fungal strains [30], and benzimidazoles from the nano-zirconia route were also tested for antifungal activity [28]. Design with reference to the ergosterol pathway is possible [15,16], but target engagement must be confirmed experimentally.

5.4 Structure–activity trends

Across reviews, C-2 substitution is the main handle for potency, N-1 substitution controls lipophilicity, and halogen, nitro, or other lipophilic electron-withdrawing groups at C-5/C-6 are frequently associated with improved antimicrobial activity [7,14]. These trends are drawn from series tested under different conditions, so they should be treated as hypotheses for design rather than fixed rules. Table 2 summarises representative examples.

Table 2. Representative examples relevant to green synthesis and antimicrobial activity.

Area	Example	Reported outcome
Antibacterial and antifungal	Benzimidazoles from a reusable nano-ZrO ₂ catalyst [28]	Gram-positive, Gram-negative, and antifungal activity reported as satisfactory
Antifungal	Bis-benzimidazoles [30]	MIC values in the low $\mu\text{g/mL}$ range against several fungal strains
Antimicrobial SAR	Reviews of benzimidazole antimicrobials [6,7]	Lipophilic electron-withdrawing substituents associated with enhanced activity in several series
Green method	DES as medium and reagent [27]	Selective synthesis of 2-substituted or 1,2-disubstituted benzimidazoles with simple work-up

5.5 Standardised susceptibility testing

MIC values are comparable between studies only when the same method, medium, inoculum, and quality-control strains are used. Broth dilution methods standardised by the Clinical and Laboratory Standards Institute (CLSI) are the reference approach [31], and detailed protocols for agar and broth dilution are available [32]. Reporting MIC rather than zone of inhibition alone, with a standard drug as control, makes data easier to compare. Docking can help rationalise activity but does not establish it.

6. Challenges and Limitations

- Green claims are often not supported by quantitative metrics such as E factor, energy use, or catalyst reuse data [17].
- Antimicrobial assays vary in method, organisms, and reporting, which limits comparison between series [31].
- Mechanism and target validation are frequently missing, and cytotoxicity against mammalian cells is not always reported, so selectivity is hard to judge.
- Planar aromatic derivatives can have limited solubility and permeability, which should be assessed alongside potency [33].
- Compounds that are potent against susceptible strains may lose activity against resistant isolates, so resistant strains should be included where possible [1,3].
- Microwave, mechanochemical, and nanocatalytic methods need demonstration at larger scale, and catalyst reuse claims should be verified.

7. Proposed Strategy for Green Synthesis and Antimicrobial Evaluation

7.1 Design and green synthesis

A rational design starts from a 2-substituted or N-substituted benzimidazole core and varies substituents at C-2, N-1, and C-5/C-6. The synthetic route should be chosen on measurable green criteria (solvent, energy, catalyst reuse, E factor) [17,18]. Identity and purity should be confirmed by infrared spectroscopy, proton and carbon-13 NMR, and mass spectrometry, and residual catalyst or acid should be excluded before biological testing.

7.2 Stepwise antimicrobial evaluation

Table 3 sets out a stepwise framework in which compounds are advanced only if they show consistent activity, acceptable cellular tolerability, and suitable physicochemical properties.

Table 3. Proposed stepwise evaluation of green-synthesised benzimidazoles.

Stage	Methods	Purpose or readout
1. Green synthesis and characterisation	Green route with recorded metrics; IR, NMR, MS, purity check	Structure and purity confirmed; greenness documented
2. Primary antimicrobial screen	Broth microdilution MIC against Gram-positive, Gram-negative, and fungal strains, including resistant isolates [31,32]	Activity and spectrum; comparability with other studies
3. Selectivity	Cytotoxicity assay in mammalian cell lines	Activity relative to host-cell toxicity
4. Mechanism	Target-specific assays where a target is proposed	Mechanism beyond phenotypic activity
5. Physicochemical properties	Solubility, permeability, metabolic stability [33]	Drug-likeness and decision to progress
6. In vivo	Efficacy and safety in suitable infection models	Only for compounds with consistent activity and an acceptable profile

8. Conclusions and Future Perspectives

- Green methods such as water, solvent-free and mechanochemical conditions, microwave and ultrasound activation, deep eutectic solvents, and reusable catalysts offer cleaner routes to benzimidazole derivatives.
- Antimicrobial activity depends on the structure and purity of the final compound; the green route contributes mainly to sustainability and cleaner products.
- Antibacterial and antifungal benzimidazoles show promising activity, but data are limited by assay variability and incomplete mechanistic information.
- Future work should report green metrics, use standardised MIC methods with resistant isolates, assess cytotoxicity and physicochemical properties, and validate mechanisms.

Abbreviations

AMR, antimicrobial resistance; CLSI, Clinical and Laboratory Standards Institute; DES, deep eutectic solvent; IR, infrared; MIC, minimum inhibitory concentration; MS, mass spectrometry; NMR, nuclear magnetic resonance; SAR, structure–activity relationship.

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Conflict of Interest

The authors declare that they have no known financial, commercial, or personal conflicts of interest that could have influenced the work reported in this

review article. All authors confirm that the research was conducted independently and that no external organization or individual had any role that could bias the content or conclusions of this manuscript.

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