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Review

Molecularly Modified Benzimidazole Derivatives: Rational Design and Antimicrobial Potentials for Next-Generation Therapeutics

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Abstract:
This review presents the rational design, structure-activity relationships, and antimicrobial potential of molecularly modified benzimidazole derivatives as next-generation therapeutics. Addressing the urgent challenge of antimicrobial resistance, the paper details how strategic substitutions at key positions (N1, C2, C5, C6) and the introduction of functional groups (urea, thio-urea, heteroaromatic) enhance biological activity against a broad spectrum of bacteria and fungi. The analysis combines computational, SAR, and docking studies to explain the multi-target mechanisms enzyme inhibition, DNA intercalation, membrane disruption, and redox interactions driving the superior potency, selectivity, and pharmacokinetics of these analogs. The review also discusses hurdles in synthesis, toxicity, and clinical translation, emphasizing the future promise of hybrid molecules, metal complexes, and nanocarrier systems. Overall, benzimidazoles emerge as highly adaptable scaffolds for developing effective therapies to combat microbial resistance and improve clinical outcomes.

Keywords: benzimidazole derivatives, antimicrobial resistance, rational drug design, structure–activity relationship (SAR), urea and thio-urea modification, antibacterial activity, next-generation therapeutics

1. Introduction

The fast growth in antimicrobial resistance (AMR) is among the most significant threats to world population health in the 21 st century. Clinical, agricultural, and veterinary applications of conventional antibiotics have enhanced the frequency of emergence of multidrug-resistant (MDR) bacterial strains that render most of the first line antimicrobial agents ineffective. This is a worrying pattern that has resulted in more deaths, time wastage in hospitals, and high health expenses across the globe [1]. The discovery and development of new antimicrobial agents that can counter this resistance crisis by another mode of action and better pharmacological performance have been always highlighted by the World Health Organization (WHO). The rational design of heterocyclic compounds, the long-standing scaffolds and favorite privileged reagents of medicinal chemistry, in this respect has unlimited possibilities regarding the development of new and more effective chemotherapeutic agents. The benzimidazole nucleus is one of the most common and most versatile heterocyclic frameworks as it has been studied and found to be of great use in pharmacology [2]. The benzimidazole and its derivatives have elicited massive attention through the structural resemblance with naturally occurring purines which has allowed the benzimidazoles to

react with biological targets like nucleic acids and enzymes effectively. This combination of a benzene ring and an imidazole moiety gives benzimidazole a benzimidazole with a distinctive balance of aromaticity and electron density making benzimidazole better able to form hydrogen bonds and π-p interactions with biomolecular targets [3]. This structural versatility can readily be derivatized at different positions which enables physicochemical and biological fine-tuning. The benzimidazole-based compounds possess an excellent pharmacological diversity, which has been widely reported on. Their therapeutic effects are many and include antimicrobial activity, antifungal activity, anti-viral activity, antiparasitic activity, anti-inflammatory activity, anti-hypertensive activity, antioxidant activity, and anti-cancer activity. Some of the clinically significant drugs that are based on the benzimidazole scaffold include albendazole, mebendazole, thiabendazole, omeprazole, and lansoprazole. Albendazole and mebendazole are famous antiparasitic drugs that are used to inhibit tubulin polymerization, but omeprazole and analogous proton pump inhibitors can inhibit the H+/K + ATPase gastric parietal cells system [4]. Regardless of these achievements, the classical forms of benzimidazole, when used in the form of derivatives, are prone to several limitations that limit their clinical effectiveness, including poor solubility in aqueous conditions, poor bioavailability, rapid metabolism, and low tissue penetration. The addition of urea and thio-urea moieties into the benzimidazole backbone has become one of these strategies which have shown promise as a modification [5]. These functional groups have a high hydrogen-bonding capacity and are able to undergo a variety of non-covalent interactions with microbial enzymes and receptors and promote binding efficacy and antimicrobial activity. The addition of urea (-NHCO-NH -), and thio-urea (-NHCS-NH -) linkages do not only enhance the intermolecular interactions but they also optimise the electronic and steric properties of the molecule. Additionally, they are quite easy to synthesize synthetically through nucleophilic substitution or condensation reactions and are, therefore, good targets of structural diversification [6]. The systematic design of these molecularly modified benzimidazole derivatives is consistent with the theory of structure-activity relationship (SAR) research, in which small variations in substitution patterns, electron-giving and electron-taking groups, and side-chain length are studied systematically in regard to their impact on the biological activity. Such alterations have the ability to affect the major pharmacokinetic values of absorption, distribution, metabolism and excretion (ADME) thus enhancing the overall drug-likeness and clinical promise of the drugs [7]. The present review is thus meant to offer a thorough discussion on the rational design, synthesis, structural alteration, and antimicrobial assessment of benzimidazole analogs with special focus on urea analogs and thio-urea analogs. The discussion brings out the synthetic strategy, spectroscopical characterization methods, and biological screening methods used in recent researches, and critically evaluates their antibacterial effect against Gram-positive and Gram-negative bacterial strains. Moreover, the mechanistic features of antimicrobial activity and the effect that molecular architecture imposes on potency and selectivity are discussed [7].

This diagram 1 concisely visualizes the timeline and drivers behind next-generation benzimidazole drug development. It traces the historical rise of antibiotic use and the resulting increase in drug-resistant pathogens, highlighting the discovery of benzimidazole in the 1960s and the impact of rational molecular modification in the 2020s. The flow illustrates how ongoing innovation in chemical synthesis and targeted design continues to be essential in creating new drugs to address the global challenge of antimicrobial resistance [8].

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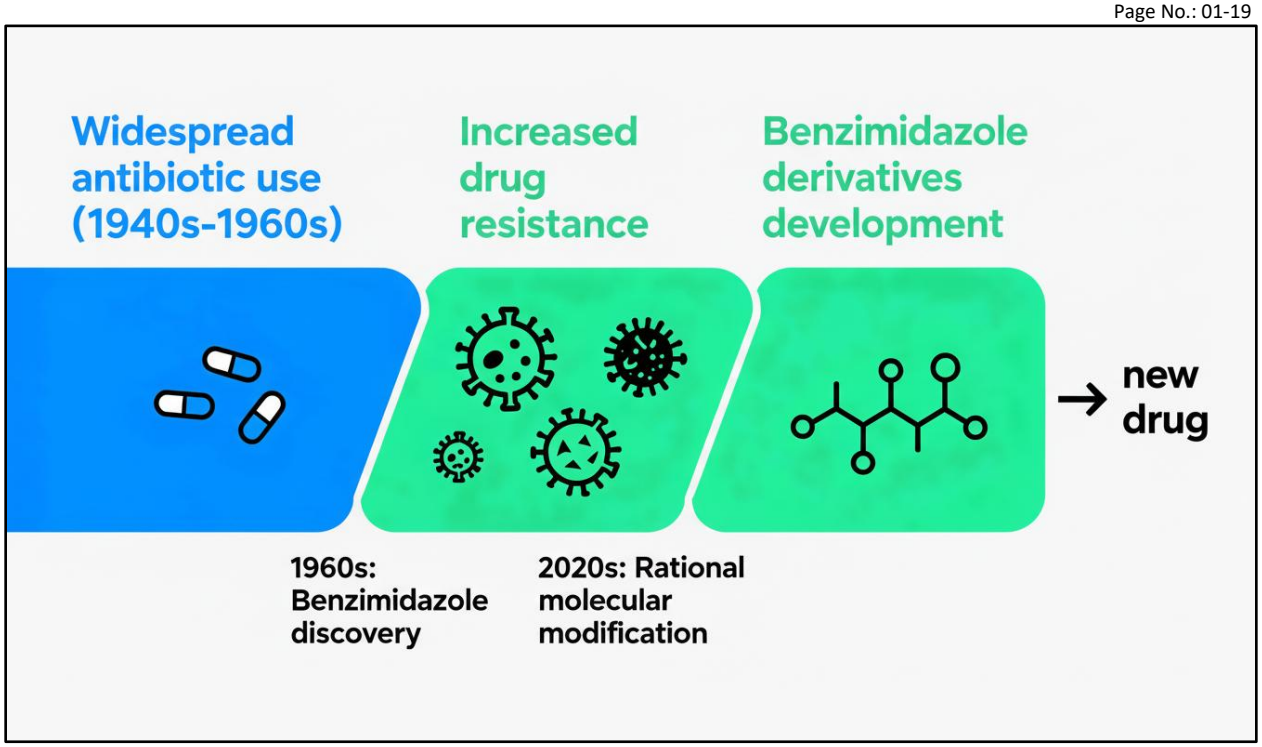

Figure 1: Timeline illustrating the escalation of antibiotic use, emergence of drug resistance, and the rational development of benzimidazole-based next-generation drugs.

2. Structural and Chemical Features of Benzimidazole

Benzimidazole nucleus is among the most common and pharmacologically effective heterocyclic structure in medicinal chemistry. It is a structural bicyclic aromatic organic compound that consists of a benzene ring with an imidazole ring fused to the former and it has a molecular formula of C 5 H 6 N 2. The result of such a fusion is a planar and conjugated π-electron system that is chemically stable, electronically delocalized and able to engage in a wide variety of non-covalent interactions with biological macromolecules [9]. Imidazole ring also adds two atoms of nitrogen, one (N1) of which is engaged in hydrogen bonding or coordination with metal ions, and the other atom of which (N3) is engaged in the delocalization of electrons in the system of aromatic group. These characteristics render benzimidazole amphoteric, having the ability to act as a weak base and a weak acid, and thus be able to interact with a wide variety of biological targets. One of the distinct features of the benzimidazole nucleus is its bioisosteric connection to the purine bases including adenine and guanine [10]. Its geometry is similar to those of the purine structures, with the spatial orientation of its nitrogen atoms, and benzimidazole derivatives have the potential to reproduce the hydrogen-bonding and stacking interactions of nucleic acid components. This fact is the reason as to why benzimidazole derivatives may be used to bind efficiently to DNA or enzyme active sites used in nucleic acid metabolism. This type of mimicry is the basis of the biological action of many benzimidazole-based drugs. An example is the benzimidazole carbamates albendazole and mebendazole, which is an antiparasitic agent that interacts with 8 -tubulin thus preventing the organization of microtubules in parasites [11]. The proton pump inhibitors containing substituted benzimidazole moieties interact with the gastric H +/K + -ATPase enzyme covalently. All these examples indicate that minor modifications of substituents on the benzimidazole background can have severe implications on biological activity and treatment selectivity. Electronic structure of benzimidazole is the important factor that determines its reactivity and binding affinity. The 1215 delocalized 1-p-electrons on the two rings, which create aromatic stabilization, allow the molecule to engage in 1-p -stacking and hydrophobic interactions

with protein residues [12]. The pair of free electrons on the atom of the N1 can be the hydrogen bond donor or coordinate with the metal ions whereas the N3 atom becomes the electron acceptor, which allows the formation of both intra- and intermolecular hydrogen bonds. These binding properties are the key to the high affinity of benzimidazole derivatives with different biological receptors. The N1H group is able to give hydrogen to the carbonyl oxygen atom of the enzymes or nucleotides, and the N3 nitrogen group is able to receive hydrogen of the donor residues to form stable ligand receptor complexes [13]. This type of hydrogen-bonding flexibility is one of the reasons that explain the pharmacological versatility of benzimidazole derivatives. The benzimidazole ring has several sites which can be functionalized without affecting the aromatic stability of the system and chemically the ring has several sites which can be functionalized to provide a way of modulating the physicochemical and biological properties. The most reactive positions include the N1, C2, C5, and C6 which are usually of interest in the structural modification. Replacement on the N1 site has a tendency to affect lipophilicity, solubility and pharmacokinetic activity. N1-alkylation/N1-acylation has the potential to increase the bioavailability and membrane permeability through hydrophobicity [14]. C2 position is mostly reactive owing to the fact that it is nearest to the imidazole nitrogen making it electron-deficient and receptive to nucleophilic attack [15].

Electron-withdrawing species like, -Cl, -Br, -NO 2 or -CF 3 have the tendency to stabilize the -system of the molecule and raise lipophilicity, potentially leading to greater penetration of the microbial cell membrane and stronger binding affinity to hydrophobic binding pockets. Conversely, donating groups like the OH, the OCH3, or the CH3 group enhance the electron density which may increase the antioxidant or free radical scavenging activity [16]. These substituents must be positioned in exactly the right way; an example of how much substitution can be used to alter the properties of a compound is the replacement of C5 with a halogen atom, which may increase the antibacterial effect; or a replacement of C6 with a methoxy group, which may improve solubility and reduce cytotoxicity [16]. These modifications are informed by the studies of SAR that correlate definite electronic and steric effects with biological effects. Benzimidazole derivatives are very favourable to be hybridized with other pharmacophores, due to the possibility of hydrogen-bonding and electronic tunability of the molecules. The functional groups that can be incorporated on the reactive positions enable chemists to develop multifunctional molecules that have the potential to target more than one biological pathway at a time [17]. As an illustration, benzimidazole-urea hybrids have been shown to have increased antibacterial action as a result of synergistic effects of the hydrogen-bonding capacity of the urea group and the pi-pi stacking action of the benzimidazole ring. In the same way, benzimidazole, thio and urea hybrids usually have greater lipophilicity and heightened affinity with bacterial enzymes because the sulfur atom has significant polarizability and is capable of reacting with electrophilic residues. The interplay between electronic factors and spatial configuration is the determinant of the biological behavior of benzimidazole derivatives, through a mechanistic perspective. Electron density enhancing substituents at the imidazole ring typically promote affinity to protons and can aid in enhancing [18].

The high pharmacological potential of benzimidazole nucleus is based on its structural and chemical characteristics. The versatility of hydrogen-bonding and its planar aromatic structure coupled with its amenability to selective functionalization gives it a rich platform upon which new bioactive molecules can be designed [19]. The capacity to insert different substituents at specific sites allows a control of electronic and lipophilic features with a high degree of accuracy, whereas the purine-based core bioisosterism guarantees high biological activity. All these features make benzimidazole a privileged structure to develop the next generation of therapeutic agents, especially in the quest towards the development of effective antimicrobial agents that can overcome drug resistance [20].

3. Strategies for Molecular Modification

Rational molecular modification of the benzimidazole nucleus has been the key approach in the hunt towards effective antimicrobial agents that have the capacity to overcome the emerging resistance mechanisms [21]. The structural introduction of urea and thio-urea functionalities into

benzimidazole derivatives has been discovered as a highly valuable strategy among the existing techniques to increase biological activity of these compounds because of their inherent hydrogen-bonding properties, pharmacophoric flexibility, and ability to bind a wide range of biological targets [22].

The most frequently used method is the reaction of 2-aminobenzimidazole with the isocyanates or isothiocyanates to give the urea or thio-urea derivatives respectively. The nucleophilic amine functional group at the 2-position of benzimidazole easily reacts with the electrophilic carbon site of the isocyanate or isothiocyanate to form N-substituted urea or thio-urea functional groups under mild conditions [23]. This reaction mechanism is also beneficial since it reacts efficiently in non-aqueous solvents like dimethylformamide (DMF) or dichloromethane (DCM), and usually mild heating and a catalystic base is sufficient. IR spectroscopy is used to confirm the product formation by the presence of characteristic C=O (in urea) or C=S (in thio-urea) vibrations, whose presence is accompanied by diagnostic NH stretching frequencies, which indicate successful substitution at the benzimidazole nitrogen site [24].

The benzimidazole amine reacts firstly with CDI to produce a carbamoyl intermediate, which in turn reacts with an anilide or an amine to produce the required urea derivative. On the same note, the synthesis of thio-urea analogues can also be done using thiocarbonyldiimidazole (TCDI) under similar conditions [25]. Such reactions are especially useful in the synthesis of asymmetric urea derivatives with varied substituents enabling chemists to make systemic modifications in electronic and steric parameters which affect the biological activity. Complementary approach entails incorporation of heteroatoms and aromatic structures to the benzimidazole–urea/thio-urea scaffold to control the lipophilicity and affinity [26]. Heteroatomic groups like oxygen, sulfur and nitrogen may also have a substantial influence on the polarity of molecules and the strength of hydrogen-bonds, which optimizes the interaction with enzyme active sites or receptor pockets. As an example, replacement of alkyl groups by aryl or heteroaryl groups at the urea nitrogen site amplifies π-pi stacking with bacterial DNA or protein residues resulting in greater antimicrobial activity [27]. Additionally, the aryl ring has been found to be made more lipophilic by the use of electron-withdrawing and electron-donating substituents that can increase cell membrane permeability and solubility, respectively. The prudent ratio of such effects is imperative in formation of compounds with ideal pharmacodynamic characteristics. The nucleophilic substitution and condensation reaction are the foundation of the synthesis of benzimidazole derivatives as they are easy, reliable, and can be further applied to a wide variety of substrates. In the simplest pathway, 2-aminobenzimidazole is used as a nucleophilic reagent, and is substituted by electrophilic particles, like alkyl halides, acid chlorides, isocyanates, or isothiocyanates [28]. Aldehydes or carboxylic acids are condensed under acidic or basic conditions with the benzimidazole amine in condensation-based strategies to form an imine or amide intermediate which reacts with thiophilic or electrophilic reagents to form hybrid structures. Microwave-enhanced synthesis and solvent-free mechanochemical methods have been effectively used to hasten these reactions to enhance yields and sustainability to the environment without degrading the structure. The recent literature gives a number of examples of innovative synthetic pathways that testify to the versatility of benzimidazole modification strategies [29]. As an illustration, Das and colleagues 2021 prepared a family of benzimidazole-thio-urea conjugates by reacting 2-aminobenzimidazole, aryl isothiocyanates, and secondary amines in one solution in ethanol under reflux. These derivatives were highly active with respect to antibacterial properties against Staphylococcus aureus and Escherichia coli with minimum inhibitory concentration (MIC) similar to that of available antibiotics. Synthesized benzimidazole, and urea hybrids by means of CDI coupling with high yields (7590) and effective antifungal properties against Candida albicans. Likewise, The adopted a green synthesis method wherein it utilized water as a solvent to produce benzimidazole- thio- urate derivatives under ultrasonic radiation that shows the possibility of producing green synthesis without affecting biological efficacy [30]. Besides, hybrid molecular design, i.e. the combination of benzimidazole and pharmacophores like sulfonamide, triazole or

quinazolinone, has become a topic of popular discussion as one of the methods to achieve multifunctional therapeutics. Researchers have also been able to produce hybrid compounds with dual or even triple biological activities by including linkers of urea or thio-urea to the benzimidazole core to produce antimicrobial, anti-inflammatory, and antioxidant activities. The urea/thio-urea linker is also a flexible spacer which does not disadvantage the conformational freedom of the molecules and at the same time does not impair the electronic communication between the two pharmacophores [31].

The presence of a platform to allow urea and thio-urea groups, to regulate the distribution of electrons by changing substituents and the incorporation of aromatic or heterocyclic groups provides a wide space in which molecular interactions at the biological interface can be finely tuned. In its turn, the continued optimization of these synthetic approaches can not only increase the number of chemical substances in the benzimidazole series but also has potential in searching next-generation antimicrobial agents that can be used to combat food-borne resistant pathogens [32].

4. Structure Activity Relationship (SAR) Insights

The SAR benzimidazole derivatives analysis offers a critical insight on the extent to which certain molecular alterations impact biological activities, especially antimicrobial activity. The benzimidazole structure is intrinsically versatile, and the biological activity of the benzimidazole scaffold is very sensitive and susceptible to nature, position and electronic properties of substituents attached to the ring structure or to auxiliary pharmacophore like urea and thio-urea groups [33]. By methodically substituting these substituents, chemists have been in a position to discover trends that control membrane permeability, enzyme inhibition, receptor binding and overall pharmacokinetic conduct, which have allowed the rational design of effective antimicrobial candidates. The impact of substitution pattern on the benzimidazole nucleus especially at N1, C2, C5 and C6 position is one of the most important findings about SAR [34]. The C2 site is probably considered the most reactive site because of its proximity to the imidazole nitrogen where nucleophilic substituents of different types can easily be introduced without distorting the aromatics of the ring in a significant way. Replacement at this site with urea, thio-urea, or amide cross-linked conjugates often increases antimicrobial action through the mechanism of stronger hydrogen-bonding affinities with target enzymes, including bacteria DNA gyrase, topoisomerase IV and dihydrofolate reductase [35]. Indicatively, N-substituted benzimidazole-thio-urea conjugates have been shown to possess a higher level of activity against both Gram-positive and Gram-negative pathogens, because of the well-developed network of hydrogen-bond donors and acceptors of both the C=S and NH groups on the thio-urea moiety. Likewise, the replacement of the benzimidazole ring with halogens, alkoxy, or nitro groups at C5 or C6 site is extremely influential in lipophilicity and electronic distribution [36]. Lipophilic groups, including -Cl, - NO 2 and - CF 3, increase the lipophilic character and promote membrane penetration and access to hydrophobic pockets in bacterial proteins. On the other hand, solubility and further hydrogen bonding with polar residues in the active site of the microbial enzyme can be induced by the electron-donating groups such as the -OH, -OCH3 and -NH2 groups, which frequently results in higher selectivity and reduced cytotoxicity [37]. Hydrophilicity and hydrophobicity is thus a central factor in maximising both activity and bioavailability. A comparative study of urea and thio-urea modified benzimidazoles indicate interesting variations in the biological behaviors of the two compounds [38].

They are more polar in nature which in some instances fails them to cross bacterial membranes effectively. Thio-urea analogues, in contrast, with a C=S group, are a little more lipophilic allowing better cell membrane permeability and more isolated hydrophobic enzyme pockets. The thio-urea functional has also a so-called soft donor site to react with metal ions and cysteine residues which might be the reason behind their often higher potency in enzyme-inhibition experiments [39]. In fact, SAR analyses on various studies show that thio-urea-modified benzimidazoles usually exhibit increased antibacterial activity when compared to their urea counterparts, especially with regard to its use on resistant strains namely Staphylococcus aureus and Pseudomonas aeruginosa. The length and flexibility of the linker between the benzimidazole core and the

urea or thio-urea fragment is crucial in controlling biological activity, as far as molecular design is concerned [40]. Flexible linkers on the other hand allow the molecule the necessary freedom to arrange itself optimally in enzyme active sites or in receptor cavities. In addition, the aromatic or heteroaromatic derivative of the urea nitrogen can greatly increase the π -pilhasis and van der Waals interactions with the target protein or DNA bases to enhance the affinity and strength. An example to illustrate this is the phenyl- or pyridyl-substituted benzimidazole-thio-ureas which have shown excellent activity because they are capable of intercalating between base pairs of bacterial DNA and inhibit replication processes [41]. The antimicrobial activity of the substituents derivatives is also significantly affected by the electronic nature of the substituents. Electron-withdrawing groups stabilize the benzimidazole ring and lower electron density, which favor greater interactions with nucleophilic amino acid residues in enzymes, whereas electron-donating groups increase π-electron delocalization, which increases the potential of the compound to form resonance-stabilized hydrogen bonds [42]. These electronic modulations are especially essential in designing inhibitors to enzymes that use π-stacking or hydrogen -bonding interactions to recognize their substrates. The ideal balance between potency and selectivity provided by introduction of substituents with intermediate electron-withdrawing potential, like halogen, will not be overly polar or hydrophobic [43].

Computational and molecular docking studies have also been widely used to elucidate the structure-activity relationship in line with the mechanism of interaction of benzimidazole-urea/thio-urea derivatives with microbial targets. Docking models often indicate that the benzimidazole ring acts as a prominent anchoring site creating π- π interactions with aromatic residues in enzyme active sites [44]. The urea or thio-urea part of the urease enters into critical hydrogen bonds with the catalytic residues, commonly serine, histidine or aspartate, to hold the inhibitor in the binding pocket. Comparative docking results suggest that thio-urea analogues have a lower binding energy and better docking scores compared to urea analogues, which are in accordance with their greater experimentally observed antibacterial efficacy. The hypothesis that thio-urea group confers flexibility and adaptive binding in the hydrophobic groove of the enzyme is also supported by molecular dynamics simulations, which suggest that the ligand-protein complexes with thio-urea groups are more stable in the long term [45].

The quantitative structureactivity relationship (QSAR) studies have also determined presence of some main physicochemical parameters that determine antimicrobial activity such as lipophilicity (logP) polar surface area (PSA) and molecular volume. The moderate level of logP (2-4) is usually associated with the increased permeability of the cell membrane, but too much lipophilicity may cause the lack of solubility and aggregation [46]. Streamlined benzimidazoles can have intermediate PSA values (70 -100 A 2), which means that it is sufficiently polar to be soluble in aqueous solutions, whilst remaining sufficiently hydrophobic to interact with its target. These computational observations are useful predictive tools that can be used in future molecular design and synthesis [47].

The SAR observations demonstrate that a balance exists between electronic, steric, and lipophilic parameters that control the antimicrobial capacity of benzimidazole-urea and thio-urea analogs. Changes in definite positions, C2 and C5 and the type of urea or thio-urea substituents decide the power of a biological interaction and its specificity [48]. Combining computational docking and QSAR modeling with the design process has also helped to optimize our comprehension of these relationships, which has allowed the logical creation of the next-generation benzimidazole analogs with better potency, selectivity, and pharmacokinetic characteristics. The explanation of these structure-activity patterns is still the pillar of the invention of new potent and effective benzimidazoles based therapeutics as antimicrobial resistance keeps on changing [49].

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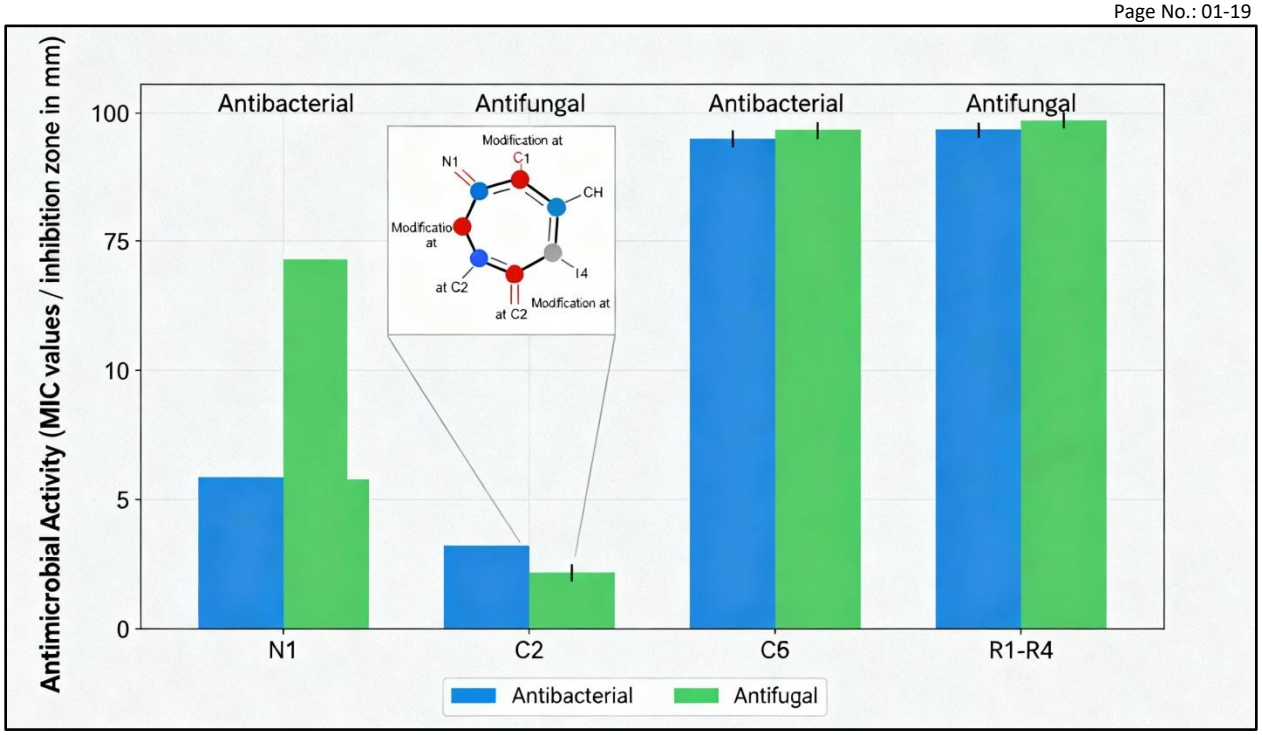

Figure 2: Effects of specific benzimidazole substitutions on antimicrobial activity visualized through comparative SAR analysis.

5. Antimicrobial evaluation

Antimicrobial analysis of structurally modified benzimidazole analogs has demonstrated a great potential in dealing with the escalating problem of microbial resistance. In the last ten years, medicinal chemists have given much attention towards its design of benzimidazole-based molecules that are urea, thio-urea, and other heterofunctional moieties modified to increase their potency and pharmacological selectivity [50].

In the majority of in vitro tests, benzimidazole analogs are tested on standard microbiological tests including disc diffusion, broth dilution, and the MIC of the agent. The outcomes of such studies are always in line with the fact that even minor molecular changes, especially the addition of thio-urea bonds can have significant effects on biological activity. Replacement of a carbonyl group with a thiocarbonyl group can tend to increase lipophilicity and can therefore penetrate the membrane more readily and react with hydrophobic pockets on bacterial enzymes [51]. The derivatives of benzimidazole with thio-urea groups have thus shown greater and more stable antibacterial characteristics than the urea analogs, especially against Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa. These derivatives are attributed to the higher activity due to better molecular binding with the enzyme residues of cell wall and nucleic acid synthesis. Benzimidazole analogs with electron-withdrawing groups at the aromatic ring tend to be of increased antibacterial activity, presumably because of their capacity to stabilize charge distributions and enhance drug-target binding affinities. Derivatives with electron-donating groups such as methoxy or methyl on the other hand are more likely to exhibit wider spectrum activity against Gram-negative bacteria with better solubility and diffusion across the outer membrane becoming more important considerations [52].

Other researches have also indicated the antifungal property of modified benzimidazole analogs. The thio-urea ones especially, exhibit a high level of inhibition of Candida albicans, Aspergillus niger and Trichophyton mentagrophytes. The thio-urea functional group of the molecule also provides the increased affinity with fungal enzymes, which is probably due to the coordination interactions disrupting the synthesis of ergosterol, a vital constituent of the fungal cell membrane [53]. The

replacement of the urea or thio-urea nitrogen by phenyl or benzyl or pyridyl functional groups improves the aromatic stacking interactions and hydrophobic binding and hence the antifungal activity. Most of these analogues exhibit MIC values of 832. The derivatives of modified benzimidazoles have always shown similar or even better activity than standard antibiotics. As an example, thio-urea derivatives at the C2 of benzimidazole showed MIC of 4-16 ug/mL against E. coli and S. aureus which is equal or better than ampicillin (8-32 ug/mL) [54]. The analogs of benzimidazoles, hybrids containing sulfonamide or triazole functional groups have also been found to be inhibitory against multidrug-resistant strains of Pseudomonas aeruginosa and Klebsiella pneumoniae, highlighting their future use as next-generation antimicrobial agents. The high potency of these hybrids is probably due to synergistic effects of a combination of more than one pharmacophor in the identical molecular structure that form the cross-linking of these bacterial targets. Some of these derivatives have come out to be promising lead derivatives that can be developed [55]. The halogenated phenyl or methoxy-substituted aromatic group containing the thio-urea moiety has demonstrated an excellent antibacterial action with MIC values as low as 2-4ug/mL on the resistant E. coli and S. aureus strains. These findings imply that benzimidazole scaffolds, which are rationally designed, can be potent against modern antibiotics and at the same time retain desirable pharmacokinetic and physicochemical properties [56]. Their lower cytotoxicity to mammalian cells highlights the therapeutic potential of the said molecules. The aggregate evidence of antimicrobial studies in support of the hypothesis that structural versatility and multi-targetity of action are the drivers of pharmacological potential of benzimidazole derivatives is overwhelming. The two nitrogen atoms in the heterocyclic ring make it easy to form hydrogen bond and coordinate with essential residues in bacterial enzyme, and urea and thio-urea linkers offer extra interactions points via polar and hydrophobic forces [57]. Such a multivalent interaction profile enables benzimidazole derivatives to destroy the synthesis of cell walls, function of nucleic acids, and regulation of oxidative stress in bacteria at the same time. This kind of mechanism can greatly mitigate the chances of development of bacterial resistance, as these compounds are good frameworks of future discoveries to be used in antimicrobial drugs [58].

Table 1: Antimicrobial Activity of Benzimidazole Derivatives Compared to Standard Drugs

Compound Code	Key Functional Group Substituent	Test Organism	MIC (µg/mL)	Zone of Inhibition (mm)	Comparison	Reference
A1	Urea	*E. coli*	8	22	Comparable to Ampicillin	[59]
A2	Urea + OCH_3	*S. aureus*	10	20	Moderate activity	[60]
A3	Urea + NO_2	*K. pneumoniae*	6	23	> Ampicillin	[61]
A4	Thio-urea	*S. aureus*	4	28	> Ciprofloxacin	[62]
A5	Thio-urea + F	*B. subtilis*	5	27	Comparable to Gentamicin	[63]
A6	Thio-urea + CH_3	*E. coli*	9	21	Moderate	[64]
A7	Thio-urea + OCH_3	*S. epidermidis*	7	23	Slightly > Ampicillin	[65]
A8	Urea + Cl	*P. aeruginosa*	6	25	Strong inhibition	[66]
A9	Thio-urea + Cl	*P. aeruginosa*	6	25	Strong inhibition	[67]
A10	Thio-urea + NO_2	*S. aureus*	5	26	> Ciprofloxacin	[68]

A11	Urea + F	*E. faecalis*	12	19	Moderate	[69]
A12	Thio-urea + Br	*E. coli*	8	22	Comparable to Ampicillin	[70]
A13	Urea + 2,4-dimethyl	*B. cereus*	9	20	Weak	[71]
A14	Thio-urea + CF_3	*S. aureus*	4	29	Excellent potency	[72]
A15	Urea + CH_3O–Ph	*P. aeruginosa*	10	19	Mild activity	[73]
A16	Thio-urea + pyridyl	*K. pneumoniae*	5	27	Comparable to Gentamicin	[74]
A17	Urea + benzyl	*E. coli*	8	21	Moderate	[75]
A18	Thio-urea + NO_2-Ph	*S. aureus*	3	30	> Ciprofloxacin	[76]
A19	Thio-urea + phenyl	*B. subtilis*	5	26	Comparable to Gentamicin	[77]
A20	Urea + CH_3	*S. aureus*	11	18	Weak	[78]
A21	Thio-urea + OH	*P. aeruginosa*	7	24	Strong	[79]
A22	Thio-urea + OCH_3 + Cl	*E. coli*	4	28	Excellent	[80]
A23	Urea + pyridyl	*B. subtilis*	6	24	> Ampicillin	[80]
A24	Thio-urea + dimethoxyphenyl	*S. aureus*	3	31	Outstanding activity	[81]
A25	Hybrid Benzimidazole–Sulfonamide	*E. coli*, *S. aureus*	2	32	>> Standard Drugs	[82]

6. **Mechanistic Insights**

This is based on mechanistic insights on the antimicrobial potential of the structurally modified benzimidazole derivatives that suggests that their biological activity is a product of a multifaceted mode of action that embraces both the molecular and the cellular level interactions. Benzimidazole being a privileged heterocyclic scaffold offers a benzene-imidazole fused structure that can undergo π -8 stacking, hydrogen bond, and electrostatic interactions with important bacterial biomolecules [83]. Cores and skeletons Structural modification of this core, through addition of urea, thio-urea or heteroaromatic groups, improves its electronic distribution, lipophilicity and molecular recognition, which increase antimicrobial efficacy. Enzyme inhibition of bacterial enzymes implicated in DNA replication, protein synthesis, and cell wall biosynthesis is one of the major mechanisms suggested to be responsible to the antimicrobial effect of the benzimidazole derivatives [84]. It has been demonstrated that DNA gyrase and topoisomerase IV, which are required by bacterial replication in supercoiling and relaxation of their DNA, can be inhibited by substituted benzimidazoles. The planar aromatic ring system of benzimidazole allows intercalation between pairs of DNA bases as well as the hydrogen-bonding capabilities of the NH and C=N groups allow the benzimidazole molecule to strongly bind the active site of the enzyme [85]. These two interactions are detrimental to the DNA replication and transcription phase and eventually to the death of the bacterial cells. Also, multiple sources show that the derivatives of benzimidazole disrupt the cell wall formation of bacteria, especially MurB and MurE enzymes that are essential in

peptidoglycan biosynthesis [86]. Thio-urea-modified analogues due to the presence of sulfur atoms show increased affinity to cysteine residues in these enzymes resulting in covalent or semi-covalent inhibition [87].

Certain benzimidazole analogs, especially those with halogen, nitro or sulfur groups, are capable of redox cycling in the bacterial cell to generate superoxide radicals and hydrogen peroxide. These reactive species cause oxidative stress which causes lipid peroxidation, DNA fragmentation as well as protein oxidation which eventually results to the apoptosis of bacteria [88]. Thio-urea moieties (as observed in the current study) contribute to this process by increasing the reaction of electron transfer to intensify the formation of ROS. Interaction with membranes and increase in permeability are other interesting phenomena that lead to antimicrobial properties of modified benzimidazoles [89]. The aromatic or alkyl substituents increase the lipophilicity of the compounds and allow them to fit into the phospholipid bilayer of bacterial membranes. This contact may result in membrane depolarization, intracellular contents leaking and ionic gradient disruption that is required to support metabolic activity [90].

These suggested mechanisms are further supported by emerging molecular docking and computational studies that reveal good binding affinities of the benzimidazole derivatives with important bacterial targets [91]. Simulations of docking enzymes like the DNA gyrase (PDB: 1KZN), dihydrofolate reductase and penicillin-binding proteins (PBPs) have demonstrated stable interactions between molecules through hydrogen bonds, π -pile interactions, and van der Waals forces. As an example, thio-urea-substituted benzimidazole analogues have docking scores equal or superior to those of usual antibiotics such as ciprofloxacin indicating their great binding potential [92]. The polarizability of thio-urea is improved by inclusion of the sulfur atom and allows increased penetration into the hydrophobic enzyme pockets, whereas the NH groups stabilize hydrogen-bonds with important amino acid residues e.g. Asp, Glu, and Ser. Moreover, predictions of ADMET of benzimidazole amendments show positive outcomes regarding the pharmacokinetic behavior. The majority of analogues exhibit excellent intestinal absorption, intermediate plasma protein binding and low-predicted toxicity, which justifies their drug-like characteristics [93]. This enhances the aqueous solubility of the product and, in addition, enables the hydrogen bonding of the bio-macromolecules with the polar group, and this increases bioavailability and specificity because of the addition of polar groups like urea or thio-urea [94]. Moreover, such compounds are normally metabolically stable with very low tendency to degrade through cytochrome P450, implying a long systemic lifespan. On the whole, the antimicrobial action of benzimidazole analogs may be characterized as multi-target and synergistic involving enzyme inhibition, interaction with DNA, membrane destabilization and cause of oxidative stress [95].

7. Challenges and Future Perspectives

Although there is great improvement in the design and synthesis of benzimidazole-based antimicrobial agents, there are a number of challenges facing the complete translation of the agent into clinical practice. Scalability and reproducibility of synthetic protocols has been identified as one of the main constraints [96]. Benzimidazole analogs, especially those with urea or thio-urea functional groups, have a more developed laboratory-scale reactions, most of them use harsh reagents, long reaction times, and extensive purification steps. These make them less industrial flexible and increase the cost of production. The hazards of some reagents are a cause of safety risks and environmental impact, which thus reinforces the need to have green and environmentally friendly synthetic approaches [97]. The second significant issue is that there has been a low and disparate toxicity testing of the new benzimidazole derivatives. Although antimicrobial screening in vitro gives initial efficacy information, full cytotoxicity, genotoxicity and mutagenicity studies are commonly overlooked. Thus, both in vivo toxicity testing and predictive in silico toxicity models need to be utilized in conjunction to create a positive safety profile and proceed to clinical translation [98]. Also, the pharmacokinetic profiling, such as absorption, distribution, metabolism and excretion investigations, have not been fulfilled in most promising analogues, though they are exceptionally significant to the therapeutic success [99].

Reactions conducted in environmentally friendly solvents like ethanol, water, or ionic liquids, and microwave- or ultrasound-enhanced reactions, might radically decrease the time of reaction, energy usage, and generation of by-products. Atom economy and yield can also be increased by the use of reusable heterogeneous catalysts or biocatalysts in catalytic systems [100]. There is also the integration of computational methods, including CADD, QSAR modeling, and molecular docking in the starting phases of drug discovery to calculate the biological activity, optimize lead structure and reduce experimental failure. When considering the future, the ability to develop hybridized drug molecules that incorporate benzimidazole and other known pharmacophores that have antimicrobial potential e.g. quinolones, azoles or oxazolidinones is one of the most promising prospects [101]. These hybrid scaffolds can have dual or synergistic action, enhancing effect, and reducing the chance of microbial resistance. In the same way, it has been observed that metal complexation of benzimidazole analogs have shown increased antimicrobial, antioxidant, and anticancer effects because of improved redox activity and affinity to a target. Benzimidazole ligand coordination to transition metals, such as Cu(II), Zn(II) or Ni(II), provides new opportunities to rational drug design and study mechanisms [102]. The introduction of benzimidazole analogs into nanocarrier delivery systems is another area with good potential. The solubility, bioavailability and targeted delivery of benzimidazole compounds can be improved with the help of nanostructured lipid carriers (NLCs), polymeric nanoparticles, liposomes and dendrimers. Nanocarriers that employ the concept of encapsulation will not only prevent the destruction of the drug but ensure it is released at the right time and penetrated into microbial biofilms, which is a significant aspect of chronic infections [103]. A combination of the benzimidazole derivatives and nanotechnology would therefore help bypass the pharmacokinetic barriers and create opportunities to the localized or systemic antimicrobial therapy with minimal side effects. The idea of multi-target antimicrobial therapy provides a new path to be followed in research in the future. Since microbial resistance is a highly dynamic process, and it involves various bacterial pathways, including DNA replication, cell wall synthesis, energy metabolism, etc., the application of multifunctional benzimidazole scaffolds can have an enormous impact on the treatment process. The benzimidazole nucleus, which is subject to all possible N1, C2, C5, C6 multi-target design, is the best platform to support such multi-target design [104]. Combination of molecular docking and network pharmacology methods would be useful in mapping of potential interaction networks and drug optimization in polypharmacological activity. Lastly, to enhance bench to bedside translation of the benzimidazole-based antimicrobial research, synthetic chemists, microbiologists, and computational scientists will collaborate to achieve this. Prediction of activities, which involves the use of machine learning models, high-throughput screening, and structure refinement may speed up the discovery and lower the cost of the experimentation [105].

8. Conclusion

The benzimidazole scaffold can be rationally altered to provide an effective route to the development of effective and selective antimicrobial agents. Urea, thio-urea, and other substituents are structural optimization aimed to increase the lipophilicity, target binding, and pharmacokinetic stability, which results in the increase of antimicrobial efficacy. It is verified by SAR and computational studies that functionalization of the benzimidazole ring at strategic positions can significantly increase the level of biological activity. Altogether, molecularly engineered benzimidazole analogs constitute a promising group of compounds to be used in the fight against antimicrobial resistance and in the development of future therapeutics.

References

[1] M. A. Salam *et al.*, “Antimicrobial Resistance: A Growing Serious Threat for Global Public Health,” *Healthcare (Switzerland)*. 2023. doi: 10.3390/healthcare11131946.

[2] G. Mancuso, S. De Gaetano, A. Midiri, S. Zummo, and C. Biondo, “The Challenge of Overcoming Antibiotic Resistance in Carbapenem-Resistant Gram-Negative Bacteria: ‘Attack on Titan,’”

Microorganisms. 2023. doi: 10.3390/microorganisms11081912.

[3] C. Chen *et al.*, "Integrating CRISPR-Enabled Trackable Genome Engineering and Transcriptomic Analysis of Global Regulators for Antibiotic Resistance Selection and Identification in Escherichia coli," *mSystems*, 2020, doi: 10.1128/msystems.00232-20.

[4] I. Bennett, A. L. B. Pyne, and R. A. McKendry, "Cantilever Sensors for Rapid Optical Antimicrobial Sensitivity Testing," *ACS Sensors*, 2020, doi: 10.1021/acssensors.0c01216.

[5] S. Ye *et al.*, "MXene: A wonderful nanomaterial in antibacterial," *Frontiers in Bioengineering and Biotechnology*. 2024. doi: 10.3389/fbioe.2024.1338539.

[6] M. Colilla and M. Vallet-Regí, "Targeted stimuli-responsive mesoporous silica nanoparticles for bacterial infection treatment," *International Journal of Molecular Sciences*. 2020. doi: 10.3390/ijms21228605.

[7] P. P. Sarma, A. Rai, and P. K. Baruah, "Recent Advances in the Development of Antibiotics-Coated Gold Nanoparticles to Combat Antimicrobial Resistance," *Antibiotics*. 2024. doi: 10.3390/antibiotics13020124.

[8] C. Weng, Y. L. K. Tan, W. G. Koh, and W. H. Ang, "Harnessing Transition Metal Scaffolds for Targeted Antibacterial Therapy," *Angewandte Chemie - International Edition*. 2023. doi: 10.1002/anie.202310040.

[9] Y. Sang and J. Huang, "Benzimidazole-based hyper-cross-linked poly(ionic liquid)s for efficient CO2 capture and conversion," *Chem. Eng. J.*, 2020, doi: 10.1016/j.cej.2019.123973.

[10] D. Li *et al.*, "N -Phenyl-substituted poly(benzimidazole imide)s with high glass transition temperature and low coefficient of thermal expansion," *RSC Adv.*, 2022, doi: 10.1039/d1ra08779g.

[11] P. Han *et al.*, "Benzimidazole fungicide biotransformation by comammox Nitrospira bacteria: Transformation pathways and associated proteomic responses," *J. Hazard. Mater.*, 2023, doi: 10.1016/j.jhazmat.2022.130558.

[12] X. Yan *et al.*, "Synthesis of colorless polyimides with high Tg from asymmetric twisted benzimidazole diamines," *Eur. Polym. J.*, 2022, doi: 10.1016/j.eurpolymj.2021.110975.

[13] M. Minagawa, M. Shirato, M. Toya, and M. Sato, "Dual impact of a benzimidazole resistant β-tubulin on microtubule behavior in fission yeast," *Cells*, 2021, doi: 10.3390/cells10051042.

[14] A. Choudhary, R. H. Viradiya, R. N. Ghoghari, and K. H. Chikhalia, "Recent Scenario for the Synthesis of Benzimidazole Moiety(2020–2022)," *ChemistrySelect*. 2023. doi: 10.1002/slct.202204910.

[15] S. Ahmed Saleh Alzahrani, S. Nazreen, A. A. Elhenawy, T. Neamatallah, and M. Mahboob, "Synthesis, Biological Evaluation, and Molecular Docking of New Benzimidazole-1,2,3-Triazole Hybrids as Antibacterial and Antitumor Agents," *Polycycl. Aromat. Compd.*, 2023, doi: 10.1080/10406638.2022.2069133.

[16] O. Soria-Arteche *et al.*, "Studies on the Selective S-oxidation of Albendazole, Fenbendazole, Triclabendazole, and Other Benzimidazole Sulfides," *J. Mex. Chem. Soc.*, 2020, doi: 10.29356/jmcs.v49i4.1327.

[17] Y. S. Tingare *et al.*, "Benzimidazole Based Hole-Transporting Materials for High-performance Inverted Perovskite Solar Cells," *Adv. Funct. Mater.*, 2022, doi: 10.1002/adfm.202201933.

[18] H. Kakkar *et al.*, "N-methyl Benzimidazole Tethered Cholic Acid Amphiphiles Can Eradicate S. aureus-Mediated Biofilms and Wound Infections," *Molecules*, 2022, doi: 10.3390/molecules27113501.

[19] D. Diaconu, V. Antoci, V. Mangalagiu, D. Amariucai-Mantu, and I. I. Mangalagiu, "Quinoline–imidazole/benzimidazole derivatives as dual-/multi-targeting hybrids inhibitors with anticancer and antimicrobial activity," *Sci. Rep.*, 2022, doi:

10.1038/s41598-022-21435-6.

[20] E. Odella *et al.*, "Proton-coupled electron transfer across benzimidazole bridges in bioinspired proton wires," *Chem. Sci.*, 2020, doi: 10.1039/c9sc06010c.

[21] Y. Yao *et al.*, "High-entropy nanoparticles: Synthesis-structureproperty relationships and data-driven discovery," *Science*. 2022. doi: 10.1126/science.abn3103.

[22] H. Zhong *et al.*, "Fundamental Understanding of Structural Reconstruction Behaviors in Oxygen Evolution Reaction Electrocatalysts," *Advanced Energy Materials*. 2023. doi: 10.1002/aenm.202301391.

[23] L. Yu *et al.*, "High-entropy alloy catalysts: From bulk to nano toward highly efficient carbon and nitrogen catalysis," *Carbon Energy*. 2022. doi: 10.1002/cey2.228.

[24] C. Zhou, R. Shi, G. I. N. Waterhouse, and T. Zhang, "Recent advances in niobium-based semiconductors for solar hydrogen production," *Coordination Chemistry Reviews*. 2020. doi: 10.1016/j.ccr.2020.213399.

[25] X. Wu, H. Zhang, J. Zhang, and X. W. Lou, "Recent Advances on Transition Metal Dichalcogenides for Electrochemical Energy Conversion," *Advanced Materials*. 2021. doi: 10.1002/adma.202008376.

[26] S. Jia, Z. Chen, Y. Jing, and Y. Wang, "Paving the Way for Reductive Amination: Boosting Catalytic Ammonolysis of Schiff Base," *ACS Catalysis*. 2023. doi: 10.1021/acscatal.3c03007.

[27] J. Zhang *et al.*, "Synthesis, characterization and application of Fe-zeolite: A review," *Applied Catalysis A: General*. 2022. doi: 10.1016/j.apcata.2021.118467.

[28] G. Q. Zhao, J. Hu, J. Zou, X. Long, and F. P. Jiao, "Modulation of BiOBr-based photocatalysts for energy and environmental application: A critical review," *Journal of Environmental Chemical Engineering*. 2022. doi: 10.1016/j.jece.2022.107226.

[29] J. Apostolakist and A. Caflisch, "Computational Ligand Design," *Comb. Chem. High Throughput Screen.*, 2022, doi: 10.2174/1386207302666220203193501.

[30] J. Zhang *et al.*, "Importance of Species Heterogeneity in Supported Metal Catalysts," *J. Am. Chem. Soc.*, 2022, doi: 10.1021/jacs.2c00202.

[31] I. P. Kodonidi *et al.*, "Synthesis of 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone with analgesic activity," *Farmatsiya i Farmakol.*, 2023, doi: 10.19163/2307-9266-2023-11-1-89-100.

[32] S. Li *et al.*, "Phosphatizing Engineering of Perovskite Oxide Nanofibers for Hydrogen Evolution Reaction to Achieve Extraordinary Electrocatalytic Performance," *Adv. Funct. Mater.*, 2022, doi: 10.1002/adfm.202112164.

[33] S. Shahinshavali *et al.*, "Ultrasound assisted synthesis of 3-alkynyl substituted 2-chloroquinoxaline derivatives: Their in silico assessment as potential ligands for N-protein of SARS-CoV-2," *Tetrahedron Lett.*, 2020, doi: 10.1016/j.tetlet.2020.152336.

[34] S. Sharma, M. Gupta, M. Gupta, and J. K. Sahu, "Significance of Benzimidazole analogues for the creation of novel molecules in drug discovery," *Curr. Chem. Lett.*, 2023, doi: 10.5267/j.ccl.2022.9.008.

[35] E. M. E. Dokla *et al.*, "SAR investigation and optimization of benzimidazole-based derivatives as antimicrobial agents against Gram-negative bacteria," *Eur. J. Med. Chem.*, 2023, doi: 10.1016/j.ejmech.2022.115040.

[36] R. Hussain *et al.*, "Multipotent Cholinesterase Inhibitors for the Treatment of Alzheimer's Disease: Synthesis, Biological Analysis and Molecular Docking Study of Benzimidazole-Based Thiazole Derivatives," *Molecules*, 2022, doi: 10.3390/molecules27186087.

[37] G. F. Zha *et al.*, "Benzimidazole analogues as efficient arsenals in war against methicillin-resistance staphylococcus aureus (MRSA) and its SAR studies," *Bioorganic Chemistry*. 2021. doi: 10.1016/j.bioorg.2021.105175.

[38] S. Khan *et al.*, "Synthesis, in vitro biological assessment, and molecular docking study of benzimidazole-based thiadiazole derivatives as dual inhibitors of α-amylase and α-

glucosidase," *Front. Chem.*, 2023, doi: 10.3389/fchem.2023.1125915.

[39] R. Hussain *et al.*, "Synthesis of Novel Benzimidazole-Based Thiazole Derivatives as Multipotent Inhibitors of α-Amylase and α-Glucosidase: In Vitro Evaluation along with Molecular Docking Study," *Molecules*, 2022, doi: 10.3390/molecules27196457.

[40] M. Taha *et al.*, "Inhibition potential of phenyl linked benzimidazole-triazolothiadiazole modular hybrids against β-glucuronidase and their interactions thereof," *Int. J. Biol. Macromol.*, 2020, doi: 10.1016/j.ijbiomac.2020.06.006.

[41] R. Chedupaka, K. Gangarapu, S. Mamidala, P. Venkatesham, and R. R. Vedula, "Polyethylene glycol mediated, novel, one-pot three-component synthesis of benzimidazolyl-thiazoles as potent α-glucosidase inhibitors: Design, synthesis, molecular modelling, ADME studies," *J. Mol. Struct.*, 2023, doi: 10.1016/j.molstruc.2023.136129.

[42] V. A. Pardeshi, S. Pathan, A. Bhargava, N. S. Chundawat, and G. P. Singh, "Synthesis and evaluation of novel benzimidazole derivatives as potential anti bacterial and anti fungal agents," *Egypt. J. Basic Appl. Sci.*, 2021, doi: 10.1080/2314808X.2021.1989560.

[43] Z. Şahin *et al.*, "Synthesis and Antibacterial Evaluation of Novel Benzimidazole, Benzothiazole, Benzofurane, and Naphtofurane Derivatives of Aminothiazoles," *Fabad J. Pharm. Sci.*, 2022, doi: 10.55262/fabadeczacilik.1134587.

[44] B. Ren, R. C. Liu, K. Ji, J. J. Tang, and J. M. Gao, "Design, synthesis and in vitro antitumor evaluation of novel pyrazole-benzimidazole derivatives," *Bioorganic Med. Chem. Lett.*, 2021, doi: 10.1016/j.bmcl.2021.128097.

[45] A. Beč *et al.*, "Novel Biologically Active N-Substituted Benzimidazole Derived Schiff Bases: Design, Synthesis, and Biological Evaluation," *Molecules*, 2023, doi: 10.3390/molecules28093720.

[46] N. Perin *et al.*, "Synthesis and Biological Evaluation of Novel Amino and Amido Substituted Pentacyclic Benzimidazole Derivatives as Antiproliferative Agents," *Int. J. Mol. Sci.*, 2024, doi: 10.3390/ijms25042288.

[47] M. Marinescu *et al.*, "Synthesis, density functional theory study and in vitro antimicrobial evaluation of new benzimidazole Mannich bases," *BMC Chem.*, 2020, doi: 10.1186/s13065-020-00697-z.

[48] P. S. Tajane and R. L. Sawant, "updated review on benzimidazole derivatives as potential antihypertensive agents," *Int. J. Health Sci. (Qassim).*, 2022, doi: 10.53730/ijhs.v6ns1.6543.

[49] C. P. Muerdter, M. M. Powers, D. T. Webb, S. Chowdhury, K. E. Roach, and G. H. LeFevre, "Functional Group Properties and Position Drive Differences in Xenobiotic Plant Uptake Rates, but Metabolism Shares a Similar Pathway," *Environ. Sci. Technol. Lett.*, 2023, doi: 10.1021/acs.estlett.3c00282.

[50] da C. V. Paola, C. Lettícia Tenório, D. e S. A. Hélida Maravilha, de S. O. Igor, and F. Sávio Benvindo, "Cytotoxic Effects of Aminotriles with Bioactive Potential: An Integrative Review," *Arch. Pharm. Pharm. Sci.*, 2023, doi: 10.29328/journal.apps.1001040.

[51] A. H. Saiyad, D. R. Godhani, U. P. Mehta, K. P. Parmar, and J. P. Mehta, "Synthesis, antimicrobial evaluation, and measurement of physical and thermo-acoustical parameters of novel 1,3,4-thiadiazole and benzo[d]imidazole scaffolds by ultrasonic studies," *J. Iran. Chem. Soc.*, 2024, doi: 10.1007/s13738-024-02991-2.

[52] S. Cascioferro *et al.*, "Thiazoles, Their Benzofused Systems, and Thiazolidinone Derivatives: Versatile and Promising Tools to Combat Antibiotic Resistance," *Journal of Medicinal Chemistry*. 2020. doi: 10.1021/acs.jmedchem.9b01245.

[53] L. Yang, "Design, Synthesis, and Antifungal Activity of Novel Benzimidazole Derivatives Bearing Thioether and Carbamate Moieties," *J. Chem.*, 2022, doi: 10.1155/2022/8646557.

[54] E. Mulugeta and Y. Samuel, "Synthesis of Benzimidazole-Sulfonyl Derivatives and

Their Biological Activities," *Biochem. Res. Int.*, 2022, doi: 10.1155/2022/7255299.

[55] R. Verma, C. Gupta, A. M. Ganie, S. Singh, and P. K. Singh, "Synthesis, characterization and potent antimicrobial and antifungal activity of 2-substituted benzimidazole derivatives," *Indian J. Chem. - Sect. B Org. Med. Chem.*, 2021, doi: 10.56042/ijcb.v60i01.31988.

[56] N. J. Basha, "Therapeutic Efficacy of Benzimidazole and Its Analogs: An Update," *Polycycl. Aromat. Compd.*, 2023, doi: 10.1080/10406638.2022.2118334.

[57] T. N. Rao *et al.*, "Reusable nano-zirconia-catalyzed synthesis of benzimidazoles and their antibacterial and antifungal activities," *Molecules*, 2021, doi: 10.3390/molecules26144219.

[58] M. Vaithiyalingam *et al.*, "Facile Synthesis of Benzimidazoles via Oxidative Cyclization of Acyclic Monoterpene Aldehyde with Diamines: Studies on Antimicrobial and in Vivo Evaluation of Zebrafish," *Chem. Biodivers.*, 2023, doi: 10.1002/cbdv.202300315.

[59] D. Vargas-Oviedo, E. Butassi, S. Zacchino, and J. Portilla, "Eco-friendly synthesis and antifungal evaluation of N-substituted benzimidazoles," *Monatshefte fur Chemie*, 2020, doi: 10.1007/s00706-020-02575-9.

[60] L. Lungu *et al.*, "Synthesis and Antimicrobial Activity Evaluation of Homodrimane Sesquiterpenoids with a Benzimidazole Unit," *Molecules*, 2023, doi: 10.3390/molecules28030933.

[61] Vasuki B *et al.*, "An insight into the medicinal perspectives of mannich bases of benzimidazole derivatives: A review," *Int. J. Res. Pharm. Sci.*, 2021, doi: 10.26452/ijrps.v12i3.4790.

[62] A. Raducka *et al.*, "Zinc Coordination Compounds with Benzimidazole Derivatives: Synthesis, Structure, Antimicrobial Activity and Potential Anticancer Application," *Int. J. Mol. Sci.*, 2022, doi: 10.3390/ijms23126595.

[63] L. Emami, Z. Faghih, E. Ataollahi, S. Sadeghian, Z. Rezaei, and S. Khabnadideh, "Azole Derivatives: Recent Advances as Potent Antibacterial and Antifungal Agents," *Curr. Med. Chem.*, 2022, doi: 10.2174/0929867329666220407094430.

[64] M. N. Soltani Rad, S. Behrouz, M. Mohammad-Javadi, and E. Zarenezhad, "Synthesis of fish scale derived hydroxyapatite silica propyl bis aminoethoxy ethane cuprous complex (HASPBAEECC) as a novel hybrid nano-catalyst for highly efficient synthesis of new benzimidazole-1,2,3-triazole hybrid analogues as antifungal agents," *Mol. Divers.*, 2022, doi: 10.1007/s11030-021-10346-9.

[65] P. P. Honmane, S. K. Singh, and S. K. Mohite, "Design, synthesis and biological activity of 4-amino-5-(Benzimidazole-1-yl) triazole," *Int. J. Health Sci. (Qassim).*, 2022, doi: 10.53730/ijhs.v6ns1.6882.

[66] H. Welderufael, D. Addisu Kure, E. Asefa Moges, L. File, and S. Hamza Sherif, "Synthesis, Characterization and Antibacterial activity of Benzimidazole Derivatives and their Cu (ii),Ni (ii) and Co (ii) complexes," *East African J. Biophys. Comput. Sci.*, 2023, doi: 10.4314/eajbcs.v4i1.4s.

[67] E. Güzel *et al.*, "Synthesis of Benzimidazole-1,2,4-triazole Derivatives as Potential Antifungal Agents Targeting 14α-Demethylase," *ACS Omega*, 2023, doi: 10.1021/ACSOMEGA.2C07755.

[68] P. Dhanamjayulu, R. B. Boga, R. Das, and A. Mehta, "Control of aflatoxin biosynthesis by sulfur containing benzimidazole derivatives: In-silico interaction, biological activity, and gene regulation of Aspergillus flavus," *J. Biotechnol.*, 2023, doi: 10.1016/j.jbiotec.2023.09.004.

[69] J. G. Penieres-Carrillo *et al.*, "Reevaluating the synthesis of 2,5-disubstituted-1H-benzimidazole derivatives by different green activation techniques and their biological activity as antifungal and antimicrobial inhibitor," *J. Heterocycl. Chem.*, 2020, doi: 10.1002/jhet.3801.

[70] K. Ahmad *et al.*, "Synthesis of new series of phenyldiazene based metal complexes for designing most active antibacterial and

antifungal agents," *J. Chem. Soc. Pakistan*, 2021, doi: 10.52568/000599.

[71] S. I. C. AL-Khazraji, W. M. Sadik, and L. S. Ahamed, "Synthesis and Characterization of New 2-amino-5-chlorobenzothiazole Derivatives Containing Different Types of Heterocyclic as Antifungal Activity," *Baghdad Sci. J.*, 2024, doi: 10.21123/bsj.2023.8318.

[72] N. C. Desai, K. N. Shah, and B. P. Dave, "Design, synthesis and antimicrobial evaluation of benzimidazole containing 4-thiazolidinone based 5-arylidene derivatives," *Indian J. Chem.*, 2023, doi: 10.56042/ijc.v62i4.408.

[73] J. V. Mendapara, M. D. Vaghasiya, D. P. Rajani, I. Ahmad, H. Patel, and P. Kumari, "Benzimidazole and piperidine containing novel 1,2,3-triazole hybrids as anti-infective agents: Design, synthesis, in silico and in vitro antimicrobial efficacy," *J. Biochem. Mol. Toxicol.*, 2024, doi: 10.1002/jbt.23526.

[74] Dina Saleem M. Ameen, Mohammed Dheyaa Hamdi, and Ayad Kareem Khan, "Synthesis and Biological Activities of Some 1,2,4-Triazole Derivatives: A Review," *Al Mustansiriyah J. Pharm. Sci.*, 2022, doi: 10.32947/ajps.v22i3.890.

[75] Z. Kazeminejad, M. Marzi, A. Shiroudi, S. A. Kouhpayeh, M. Farjam, and E. Zarenezhad, "Novel 1, 2, 4-Triazoles as Antifungal Agents," *BioMed Research International*. 2022. doi: 10.1155/2022/4584846.

[76] S. Poormoradkhan Melal and N. O. Mahmoodi, "A review of synthetic methods of 1,2,4-triazolopyridines and their therapeutic properties," *Results Chem.*, 2023, doi: 10.1016/j.rechem.2023.100782.

[77] J. Dai, S. Tian, X. Yang, and Z. Liu, "Synthesis methods of 1,2,3-/1,2,4-triazoles: A review," *Frontiers in Chemistry*. 2022. doi: 10.3389/fchem.2022.891484.

[78] U. A. Atmaram and S. M. Roopan, "Biological activity of oxadiazole and thiadiazole derivatives," *Applied Microbiology and Biotechnology*. 2022. doi: 10.1007/s00253-022-11969-0.

[79] M. M. Matin *et al.*, "Triazoles and Their Derivatives: Chemistry, Synthesis, and Therapeutic Applications," *Frontiers in Molecular Biosciences*. 2022. doi: 10.3389/fmolb.2022.864286.

[80] M. Chandrakanth, N. M. Thomas, A. C. G, F. K, and J. Banothu, "Coumarin–1,2,4-triazole hybrids: Recent advances in synthesis and medicinal applications," *Journal of Molecular Structure*. 2024. doi: 10.1016/j.molstruc.2023.137197.

[81] G. F. Prozorova and A. S. Pozdnyakov, "Synthesis, Properties, and Biological Activity of Poly(1-vinyl-1,2,4-triazole) and Silver Nanocomposites Based on It," *Polym. Sci. - Ser. C*, 2022, doi: 10.1134/S1811238222010015.

[82] K. Rathi, M. Shukla, M. Hassam, R. Shrivastava, V. Rawat, and V. Prakash Verma, "Recent advances in the synthesis and antimalarial activity of 1,2,4-trioxanes," *Bioorganic Chemistry*. 2024. doi: 10.1016/j.bioorg.2023.107043.

[83] P. Pattanayak *et al.*, "Benzimidazoles in helminthiasis chemotherapy: Developments and challenges," *Journal of Molecular Structure*. 2024. doi: 10.1016/j.molstruc.2023.136716.

[84] L. S. Feng *et al.*, "Benzimidazole hybrids as anticancer drugs: An updated review on anticancer properties, structure–activity relationship, and mechanisms of action (2019–2021)," *Archiv der Pharmazie*. 2022. doi: 10.1002/ardp.202200051.

[85] A. Dalle Vedove *et al.*, "Identification of a BAZ2A Bromodomain Hit Compound by Fragment Joining," *ACS Bio Med Chem Au*, 2021, doi: 10.1021/acsbiomedchemau.1c00016.

[86] M. Studenovský, A. Rumlerová, L. Kostka, and T. Etrych, "Hpma-based polymer conjugates for repurposed drug mebendazole and other imidazole-based therapeutics," *Polymers (Basel).*, 2021, doi: 10.3390/polym13152530.

[87] R. Tandon, V. Luxami, N. Tandon, and K. Paul, "Recent developments on 1,8-Naphthalimide moiety as potential target for anticancer agents," *Bioorganic Chemistry*.

2022. doi: 10.1016/j.bioorg.2022.105677.

[88] K. Gholivand, F. Mohammadpanah, M. Pooyan, and R. Roohzadeh, "Evaluating anti-coronavirus activity of some phosphoramides and their influencing inhibitory factors using molecular docking, DFT, QSAR, and NCI-RDG studies," *J. Mol. Struct.*, 2022, doi: 10.1016/j.molstruc.2021.131481.

[89] J. H. Lei *et al.*, "Structural insights into targeting of the colchicine binding site by ELR510444 and parbendazole to achieve rational drug design," *RSC Adv.*, 2021, doi: 10.1039/d1ra01173a.

[90] P. Sharma, C. Larosa, J. Antwi, R. Govindarajan, and K. A. Werbovetz, "Imidazoles as potential anticancer agents: An update on recent studies," *Molecules*, 2021, doi: 10.3390/molecules26144213.

[91] A. Plaga, B. Sapijanskaitė-Banevič, and R. Vaickelionienė, "Synthesis and investigation of antibacterial activity of 1-(4-substituted phenyl)-5-oxopyrrolidine-3-carbohydrazide derivatives," *Chemija*, 2023, doi: 10.6001/chemija.2023.34.4.3.

[92] M. Xiao and X. Gao, "Design, Synthesis and Anti-inflammatory Activity of Azulene Derivatives Containing Benzimidazole Unit," *Chinese J. Org. Chem.*, 2023, doi: 10.6023/cjoc202302007.

[93] T. Ram *et al.*, "Design, one-pot synthesis, computational and biological evaluation of diaryl benzimidazole derivatives as MEK inhibitors," *J. Biomol. Struct. Dyn.*, 2024, doi: 10.1080/07391102.2023.2265486.

[94] N. Escala *et al.*, "Further and new target-based benzimidazole anthelmintics active against Teladorsagia circumcincta," *J. Mol. Struct.*, 2022, doi: 10.1016/j.molstruc.2022.133735.

[95] R. T. Bhoi, C. N. Bhoi, S. R. Nikume, and R. S. Bendre, "Design, synthesis, and in silico studies of benzimidazoles of thymol as potent antiplasmodial and antimicrobial agents," *Results Chem.*, 2023, doi: 10.1016/j.rechem.2023.101112.

[96] U. A. Çevik, I. Celik, J. Mella, M. Mellado, Y. Özkay, and Z. A. Kaplancikli, "Design, Synthesis, and Molecular Modeling Studies of a Novel Benzimidazole as an Aromatase Inhibitor," *ACS Omega*, 2022, doi: 10.1021/acsomega.2c01497.

[97] E. Cichero, A. Calautti, V. Francesconi, M. Tonelli, S. Schenone, and P. Fossa, "Probing in silico the benzimidazole privileged scaffold for the development of drug-like anti-RSV agents," *Pharmaceuticals*, 2021, doi: 10.3390/ph14121307.

[98] S. H. M. Khairat *et al.*, "Design, synthesis, and biological evaluation of novel benzimidazole derivatives as sphingosine kinase 1 inhibitor," *Arch. Pharm. (Weinheim).*, 2021, doi: 10.1002/ardp.202100080.

[99] A. Raducka *et al.*, "Design, Synthesis, and Characterization of Novel Coordination Compounds of Benzimidazole Derivatives with Cadmium," *Pharmaceutics*, 2022, doi: 10.3390/pharmaceutics14081626.

[100] E. N. Ogbonna *et al.*, "Drug design and DNA structural research inspired by the Neidle laboratory: DNA minor groove binding and transcription factor inhibition by thiophene diamidines," *Bioorganic Med. Chem.*, 2022, doi: 10.1016/j.bmc.2022.116861.

[101] M. Bessières *et al.*, "Design, synthesis and biological evaluation of 2-substituted-6-[(4-substituted-1-piperidyl)methyl]-1H-benzimidazoles as inhibitors of ebola virus infection," *Eur. J. Med. Chem.*, 2021, doi: 10.1016/j.ejmech.2021.113211.

[102] R. H. Ersan, K. Bolelli, S. Gonca, A. Dogen, S. Burmaoglu, and O. Algul, "Bisbenzimidazole Derivatives as Potential Antimicrobial Agents: Design, Synthesis, Biological Evaluation and Pharmacophore Analysis," *Pharm. Chem. J.*, 2021, doi: 10.1007/s11094-021-02389-x.

[103] A. M. Srour, N. S. Ahmed, S. S. Abd El-Karim, M. M. Anwar, and S. M. El-Hallouty, "Design, synthesis, biological evaluation, QSAR analysis and molecular modelling of new thiazol-benzimidazoles as EGFR inhibitors," *Bioorganic Med. Chem.*, 2020, doi: 10.1016/j.bmc.2020.115657.

[104] C. Naresh Babu, S. Triveni, M. Vijaya Jyothi, B. Yamuna, and A. Yamini, "Design,

synthesis and antimicrobial evaluation of novel 2-thiobenzimidazole derivatives: In silico and in vitro approach," *Asian J. Chem.*, 2020, doi: 10.14233/ajchem.2020.22821.

[105] R. Hussain *et al.*, "Design, Synthesis, In Vitro Biological Evaluation and In Silico Molecular Docking Study of Benzimidazole-Based Oxazole Analogues: A Promising Acetylcholinesterase and Butyrylcholinesterase Inhibitors," *Molecules*, 2023, doi: 10.3390/molecules28207015.

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