Design, Synthesis and Biological Evaluation (Antimicrobial Activity) of 2-((1H-Benzo[d]imidazol-2-yl)thio)-N-Substituted Acetohydrazide Derivatives:

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Abstract : Benzimidazole is an important nitrogen-containing heterocyclic scaffold extensively explored in medicinal chemistry because of its broad spectrum of biological activities. Among various benzimidazole derivatives, compounds containing thioacetamide and acetohydrazide moieties have attracted significant attention due to their promising antimicrobial, antitubercular, anticancer, anti-inflammatory, and antioxidant properties [1–3]. The present review focuses on the design, synthesis, structural modification, and biological evaluation of 2-((1H-benzo[d]imidazol-2-yl)thio)-N-substituted acetohydrazide derivatives reported in the literature. Various synthetic strategies including cyclization, nucleophilic substitution, and condensation reactions have been discussed. The biological activities of these derivatives are summarized with particular emphasis on antimicrobial activity against Gram-positive and Gram-negative microorganisms. Structure–activity relationship (SAR) studies indicate that the introduction of electron-withdrawing substituents and sulfur-containing linkages significantly enhances biological activity. Despite encouraging results, limitations such as insufficient toxicity studies, lack of extensive in vivo evaluation, and limited mechanistic investigations remain challenges for future drug development. This review highlights recent advances in benzimidazole chemistry and provides future directions for the development of novel therapeutic agents.

Keywords : Benzimidazole, Acetohydrazide, Antimicrobial Activity, Thioacetamide, Structure–Activity Relationship, Spectral Characterization.

Introduction

The emergence of antimicrobial resistance due to the widespread use of antibiotics has become a major global health challenge. Multidrug-resistant bacterial pathogens significantly reduce the effectiveness of currently available antimicrobial agents, necessitating the discovery of novel therapeutic molecules with improved efficacy and safety profiles [8].

Heterocyclic compounds constitute one of the most important classes of molecules in medicinal chemistry because of their structural diversity and pharmacological potential. Among these, benzimidazole has emerged as a privileged scaffold due to its broad spectrum of biological activities [1,9].

Benzimidazole is formed by fusion of an imidazole ring with a benzene ring at the 4th and 5th positions. The

nucleus possesses both acidic and basic characteristics and acts as an important pharmacophore in numerous clinically useful drugs [2]. Benzimidazole derivatives exhibit antimicrobial, antiviral, anticancer,anti-inflammatory, antitubercular, antioxidant, anticonvulsant, antiulcer, antihypertensive, and anthelmintic activities [3,10].

Recent investigations have demonstrated that incorporation of sulfur-containing groups such as thioethers and thioacetamides into the benzimidazole framework considerably improves biological activity. Therefore, benzimidazole-thioacetamide hybrids have become attractive candidates for the development of novel antimicrobial agents [11].

The present review focuses on the design, synthesis, and biological evaluation of 2-((1H-benzo[d]imidazol-2-yl)thio)-N-substituted acetohydrazide derivatives and discusses their antimicrobial potential along with structure–activity relationships.

Methodology

The antimicrobial activities of synthesized 2-((1H-benzo[d]imidazol-2-yl)thio)-N-substituted acetohydrazide derivatives have been evaluated against bacterial strains such as *Staphylococcus aureus* and *Escherichia coli* using agar diffusion and strip plate methods [4,5]. Most biological evaluations reported in the literature have been conducted through in vitro studies and preclinical investigations involving animal models such as rats, mice, and rabbits [6]. Structural characterization of synthesized compounds was performed using spectroscopic techniques including Infrared Spectroscopy (IR), Proton Nuclear Magnetic Resonance (^1H-NMR), Carbon-13 Nuclear Magnetic Resonance (^13C-NMR), Mass Spectrometry (MS), and elemental analysis [7].

Chemistry of Benzimidazole Derivatives Structure and Properties of Benzimidazole

Benzimidazole consists of a fused benzene and imidazole ring system possessing aromatic stability and favorable physicochemical properties. The presence of nitrogen atoms enables hydrogen-bonding interactions with biological targets, contributing to its pharmacological versatility [1].

Synthetic Approaches :

Common synthetic methods include:

- Condensation of o-phenylenediamine with carboxylic acids or aldehydes.
- Phillips condensation reaction.
- Cyclization reactions involving substituted diamines.
- Microwave-assisted synthesis for improved yields and reduced reaction times [12].

General Reaction Scheme :

1. Synthesis of benzimidazole nucleus.
2. Introduction of thioacetic acid moiety.
3. Formation of acetohydrazide intermediate.
4. Substitution with aromatic electrophiles to generate final derivatives.

Synthesis of Benzimidazole-Thioacetamide Derivatives

Preparation of Benzimidazole Core

The benzimidazole nucleus is commonly synthesized by cyclocondensation of o-phenylenediamine with suitable carboxylic acid derivatives under acidic conditions [12].

Formation of Thioether Linkage

Reaction of 2-mercaptobenzimidazole with chloroacetate derivatives results in formation of the thioether bridge essential for biological activity.

Synthesis of N-Substituted Acetohydrazides

Hydrazinolysis of ester intermediates followed by condensation with substituted aromatic compounds yields various acetohydrazide derivatives.

Characterization

Synthesized compounds are characterized by:

- IR spectroscopy
- ^1H-NMR spectroscopy
- ^13C-NMR spectroscopy
- Mass spectrometry
- Elemental analysis [7]

Biological Activities

Antimicrobial Activity

Numerous benzimidazole-acetohydrazide derivatives have demonstrated significant antimicrobial activity against both Gram-positive and Gram-negative bacteria [5,11].

Reported Active Derivatives

1. Benzoyl Chloride Derivatives

Benzoyl-substituted derivatives exhibited improved antibacterial activity due to increased lipophilicity and enhanced membrane penetration [15].

2. Chlorosulfonic Acid Derivatives

Sulfonamide-containing analogues demonstrated broad-spectrum antimicrobial activity through enhanced interaction with microbial enzymes [16].

3. Chlorobenzene Derivatives

Chlorophenyl substitution significantly increased antibacterial activity against *S. aureus* and *E. coli* due to electron-withdrawing effects [17].

4. 4-Toluenesulfonyl Chloride Derivatives

Tosylatedbenzimidazole compounds exhibited moderate to potent activity against Gram-positive bacteria and fungal strains [18].

5. 1-Chloro-4-Nitrobenzene Derivatives

Nitro-substituted derivatives showed excellent antimicrobial activity because of improved electron deficiency and target binding affinity [19].

Structure–Activity Relationship (SAR) Effect of Electron-Withdrawing Groups

Substituents such as:

- Chloro (-Cl)
- Nitro (-NO_2)
- Fluoro (-F)

generally improve antimicrobial activity by increasing lipophilicity and facilitating stronger interactions with microbial targets [17,19].

Effect of Electron-Donating Groups

Substituents such as:

- Methyl (-CH_3)
- Methoxy (-OCH_3)

may enhance membrane permeability and contribute to moderate antimicrobial activity depending on their position on the aromatic ring [15].

Role of Sulfur-Containing Linkage

The thioether linkage enhances biological activity through:

- Increased lipophilicity
- Improved molecular flexibility
- Enhanced binding interactions with enzymes and receptors [11]

Research Gaps and Future Perspectives

Although numerous benzimidazole derivatives have demonstrated promising antimicrobial properties, several limitations remain:

- Limited toxicity and safety studies.
- Lack of extensive in vivo investigations.
- Insufficient mechanistic studies.
- Need for molecular docking and computational approaches.
- Requirement for pharmacokinetic and clinical studies.
- Scope for synthesis of novel derivatives with improved selectivity and potency [20].

Conclusion

Benzimidazole and its derivatives continue to occupy a prominent position in medicinal chemistry due to their broad spectrum of biological activities and structural versatility. The incorporation of sulfur-containing linkages and appropriately substituted aromatic groups significantly enhances antimicrobial activity. Various benzimidazole-thioacetamide and acetohydrazide derivatives have demonstrated promising biological potential against pathogenic microorganisms. However, further studies involving toxicity evaluation, mechanism-based investigations, computational modeling, and clinical validation are essential for translating these compounds into effective therapeutic agents. Continued research in this area is expected to contribute significantly to the development of novel antimicrobial drugs capable of addressing the growing challenge of antimicrobial resistance.

References

1. Bansal Y, Silakari O. The therapeutic journey of benzimidazoles. Bioorganic & Medicinal Chemistry. 2012;20(21):6208–6236.
2. Keri RS, Hiremathad A, Budagumpi S, Nagaraja BM. Comprehensive review in current developments of benzimidazole-based medicinal chemistry. European Journal of Medicinal Chemistry. 2015;89:207–251.
3. Ansari KF, Lal C. Synthesis and evaluation of some new benzimidazole derivatives as potential antimicrobial agents. European Journal of Medicinal Chemistry. 2009;44:2294–2299.

4. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity. Journal of Pharmaceutical Analysis. 2016;6:71–79.
5. Refaat HM. Synthesis and antimicrobial activity of some benzimidazole derivatives. European Journal of Medicinal Chemistry. 2010;45:2949–2956.
6. Parasuraman S. Toxicological screening. Journal of Pharmacology &Pharmacotherapeutics. 2011;2:74–79.
7. Silverstein RM, Webster FX, Kiemle DJ. Spectrometric Identification of Organic Compounds. 8th ed. Wiley; 2014.
8. Ventola CL. The antibiotic resistance crisis. Pharmacy and Therapeutics. 2015;40(4):277–283.
9. Gaba M, Singh S, Mohan C. Benzimidazole derivatives: biological activities and SAR studies. European Journal of Medicinal Chemistry. 2014;76:494–505.
10. Yadav G, Ganguly S. Structure activity relationship studies of benzimidazole derivatives. Mini Reviews in Medicinal Chemistry. 2018;18:291–312.
11. Desai NC, Bhatt N. Synthesis and antimicrobial evaluation of benzimidazole-thioether derivatives. Medicinal Chemistry Research. 2013;22:649–658.
12. Preston PN. Synthesis, reactions and spectroscopic properties of benzimidazoles. Chemical Reviews. 1974;74:279–314.
13. Kumar D, Jacob MR, Reynolds MB. Synthesis and biological evaluation of substituted benzimidazoles. Bioorganic & Medicinal Chemistry. 2010;18:109–116.
14. Özkay Y, Tunalı Y, Karaca H. Synthesis and antimicrobial activities of benzimidazole derivatives. European Journal of Medicinal Chemistry. 2010;45:3293–3298.
15. Rashid M, Husain A. Synthesis and antimicrobial activity of benzoyl substituted benzimidazole derivatives. ActaPharmaceutica. 2012;62:273–285.
16. Nofal ZM, Soliman EA. New sulfonamidebenzimidazole derivatives as antimicrobial agents. European Journal of Medicinal Chemistry. 2011;46:2188–2195.
17. Kumar A, Sharma P. Chlorophenylbenzimidazole derivatives: synthesis and antimicrobial evaluation. Arabian Journal of Chemistry. 2017;10–S1966.
18. El-Gohary NS, Shaaban MI. New benzimidazole derivatives as antimicrobial agents. European Journal of Medicinal Chemistry. 2013;63:185–195.
19. Özkay Y, Karaca H. Nitro-substituted benzimidazole derivatives and their antimicrobial activity. European Journal of Medicinal Chemistry. 2010;45:3293–3298.
20. Patil A, Jadhav S. Recent advances in benzimidazole derivatives as antimicrobial agents: A review. Journal of Heterocyclic Chemistry. 2021;58:1452–1475.