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1.1. Introduction to benzimidazole

The properties of benzimidazole and its derivatives have been studied over more than one hundred years\(^1\). However, a special interest of researchers toward this class of compounds was stimulated by the fact that 5,6-dimethylbenzimidazole is a component of vitamin B\(_{12}\)\(^2\).

Shortly after, it was established that, although vitamin B\(_{12}\) is capable of stimulating the growth of bacteria, the benzimidazole fragment and some of its derivatives suppress the bacterial growth.

![Chemical Structure](image1.png)

This discovery was followed by attempts to synthesize newer antibacterial compounds based on the benzimidazoles. As a result several benzimidazole derivatives have been successfully commercialized as potent Active Pharmaceutical Ingredients.

![Chemical Structure](image2.png)

Benzimidazole derivatives find application as promising drugs in different therapeutic categories as anthelmintics,\(^{3-7}\) anti thrombotics,\(^8\) anti psychotics,\(^9\) analgesics,\(^{10-13}\) anti hypertensives,\(^{14-16}\) fungicides,\(^{17}\) anti fungals,\(^{18}\) anti histamines,\(^{19-21}\) anti emetics,\(^{22}\) anti hyperthyroidals,\(^{23}\) anti ulcerative,\(^{24}\)
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antibiotics,\textsuperscript{(25)} anti cancer agents,\textsuperscript{(26)} MCP-I antagonists,\textsuperscript{(27)} hypoglycemic agents,\textsuperscript{(28)} adrenergic agonists,\textsuperscript{(29)} anti inflammatory agents,\textsuperscript{(30)} anti tumor compounds,\textsuperscript{(31)} anti virals,\textsuperscript{(32)} anti microbials,\textsuperscript{(33)} anti HIV agents,\textsuperscript{(34)} anti allergies\textsuperscript{(35)} and anti phytovirucides\textsuperscript{(36, 37)}.

Benzimidazol-2-thiols have received considerable attention during last few decades as they are endowed with variety of biological activities of which the most profound are anti-ulcer,\textsuperscript{(38)} anti-hypertensive activity,\textsuperscript{(39)} anti-microbial,\textsuperscript{(40)} anti-inflammatory,\textsuperscript{(41)} anti-cancer activity,\textsuperscript{(42)} anti-hyperlipidemic activities.\textsuperscript{(43)} Recently various 2-mercapto benzimidazole derivatives have been synthesized and screened for anti-fungal and anti-bacterial activity.\textsuperscript{(44, 45)}

1.2. Fungi

Currently benzimidazoles are the most important group of systemic fungicides in use for controlling the fungal diseases.\textsuperscript{(46)} Benzimidazole itself and 2-methyl benzimidazole have good effect on \textit{Candida albicans} and \textit{Aspergillus fumigatus}.\textsuperscript{(47)} Two important fungicidal benzimidazole derivatives are Thiabendazole and Carbendazim.

Fungal disorders pose a continuous and serious threat to human health and life.\textsuperscript{(48)} These fungal disorders in humans can be classified into (a) allergic reactions to fungal proteins, (b) toxic reactions to toxins present in certain fungi and (c) infections (mycoses). Healthy individuals are susceptible to a host of superficial, cutaneous, subcutaneous and in certain instances, systemic infections that cause a variety of conditions ranging from Athletes foot and nail infections to severe life-threatening disseminated disease (e.g., histoplasmosis).\textsuperscript{(49)} Many fungal infections are caused by opportunistic pathogens that may be endogenous (Candida infections) or acquired from the environment (Cryptococcus, Aspergillus infections).\textsuperscript{(48)} The other type of fungal infection, that is, invasive fungal infections and dermatomycoses are produced by fungal organisms in the individuals with increased vulnerability such as neonates, cancer patients receiving chemotherapy, organ transplant patients, and burns patients, apart from those with acquired
immunodeficiency syndrome (AIDS). Other risk factors include corticosteroid and antibiotic treatments, diabetes, lesions of epidermis and dermis, malnutrition, neutropenia and surgery.\(^{50-52}\)

In recent years, the incidence and severity of fungal diseases has increased, particularly in patients with impaired immunity. The growing number of cases of fungi involved in sepsis is a consistent trend.\(^{53}\) There is considerable alarm amongst the medical profession regarding fungal disease. Dermatophyte infections such as tinea pedis and candidiasis, although rarely fatal, are common and widespread throughout the world. Pathogens such as Candida albicans, Cryptococcus neoformans, Pneumocystis carinii and Aspergillus fumigatus are the causes of considerable morbidity and mortality in immuno-compromised patients.\(^{54}\) Aspergillus and Candida spp. account for the majority of documented infections. Recent epidemiological trends indicate a shift towards infections by Aspergillus spp., non-albicans Candida spp. and previously uncommon fungi that often have diminished susceptibility to current antifungal agents.\(^{55-59}\)

Clinically, candidiasis and aspergillosis account for between 80% and 90% of systemic fungal infections in immunocompromised patients.\(^{60}\) Although, the arsenal of antifungal drugs has expanded, currently available antifungal drugs do not meet the increasing requirements of managing infection in the complex patient populations. The development of new antifungal drugs has been constantly required in the clinical therapy.\(^{61}\)

Although these are effective against many groups of fungi, there are some major groups of fungi (e.g. Alternaria solani) which are quite insensitive to these compounds.\(^{62}\) The development of resistance to current anti-microbial therapy continues to stimulate the search for more effective agents.

1.3. **Bacteria**

The worldwide emergence of antibiotic-resistant bacteria threatens to undo the dramatic advances in human health that were ushered in with the discovery of
these drugs in the mid-1900s. Today, resistance has rendered most of the original antibiotics obsolete for many infections, mandating an increased reliance on synthetic drugs.

Resistance can arise as a result of altered molecular targets, efflux of antibiotics from within the cell, blockade of antibiotic entry into the cell, and chemical modification or destruction of the compounds. In many cases, a single antibiotic or class is impacted by more than one or even all of these mechanisms. Furthermore, these mechanisms can be collected within a single organism resulting in combinatorial resistance. The range of resistance mechanisms is common to pathogenic and nonpathogenic bacteria and suggests a complex natural history of evolution and selection. The evolution of enzymes that modify antibiotic targets and antibiotics themselves are of particular interest.\(^63\)

Bacteria also develop resistance to synthetic drugs, typically by acquiring chromosomal mutations.\(^{64-69}\) Within the classical paradigm that mutations are the inevitable consequence of replicating a large genome with polymerases of finite fidelity, resistance-conferring mutations are unavoidable. However, recent evidence suggests that bacteria may play a more active role in the mutation of their own genomes in response to at least some DNA-damaging agents by inducing proteins that actually promote mutation.\(^{70-78}\)

Chemical modification or destruction of compounds includes Phosphorylation, Acetylation, Nucleotidylation, Monooxygenation, Hydrolysis, Glycosylation, ADP-ribosylation, etc. Some bacteria produce enzymes that neutralize antibiotics by adding acetyl (COCH\(_3\)) or phosphate (PO\(_3^{2-}\)) groups to a specific site on the antibiotic.\(^{79}\) This modification reduces the ability of the antibiotic to bind to ribosomes, rendering it harmless to the cell.\(^{80}\)
Other reasons are efflux pumps, located in the cell membrane, are one method of protection that many bacteria use against the influx of antibiotics. The offensive antibiotic is pumped out of a cell that possesses these pumps before the antibiotic can cause harm to the cellular machinery. Ribosomal protection proteins (RPP) are another source of resistance bacteria use to protect themselves from antibiotics. These proteins protect ribosomes by binding them and changing their shape or conformation. The change in the ribosome shape prevents an antibiotic from binding and interfering with protein synthesis. The RPP-bound ribosomes are able to function normally during protein synthesis, an important feature of this method of antibiotic resistance.

In the past decade, there has been a dramatic increase in the occurrence of bacterial strains that are resistant to multiple classes of antibiotics; for instance, methicillin resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus faecalis (VRE) are known to cause problems particularly in hospital settings, while infections by multidrug-resistant pneumococci occur also in the community.

The increased prevalence of antibiotic-resistant bacteria heralds a need for new drugs and novel strategies to identify better drug targets. There is a demand for antibiotics acting by a novel mechanism and consequently lacking cross resistance to existing agents.

A novel benzimidazole molecule, named antibiofilm compound 1 (ABC-1), identified in a small-molecule screen, was found to prevent bacterial biofilm formation in multiple Gram-negative and Gram-positive bacterial pathogens, including Pseudomonas aeruginosa and Staphylococcus aureus.

Researchers at the Southern Research Institute screened known tubulin inhibitors against Mtb and identified several pyridopyrazine and pteridine based Filamenting temperature-sensitive mutant Z (FtsZ) inhibitors with anti-TB activity. Later, Slayden et al. found that thiabendazole and albendazole, known tubulin inhibitors, interfered and delayed the Mtb cell division processes. Taking into account the structural similarity of the pyridopyrazine moiety, pteridine
moiety, albendazole, and thiabendazole.\(^{(85-87)}\) It was envisioned that the benzimidazole scaffold would be a good starting point for the development of novel FtsZ inhibitors, which would have activity against both drug sensitive and drug resistant Mtb. Libraries of novel benzimidazoles were created through rational drug design. A good number of these benzimidazoles exhibited promising MIC values for their antibacterial activity against Mtb H37Rv strain.

Though all seven positions in the benzimidazole nucleus can be substituted with a variety of chemical entities, but most of the biologically active benzimidazole based compounds bear functional groups at 1, 2 and/or 5(or 6) positions. Accordingly, the compounds may be mono-, di- or tri-substituted derivatives of the nucleus. 1-substituted benzimidazole compounds have been found to exhibit poor antibacterial properties.\(^{(89)}\) Presumably the extensive non-polar portion of molecules mimics the correspondingly non-polar steroidal portion of the substrate for lanosterol 14α-demethylase. The non-polar functionality confers a high degree of lipophilicity to antifungal azoles.\(^{(90)}\)

### 1.4. **Tuberculosis**

Tuberculosis, which is caused by single infectious agent *Mycobacterium tuberculosis*, is one of the most important killing infectious diseases. WHO estimated that there were 8.8 million new cases of tuberculosis in 2002, of which 3.9 million were smear-positive. The global incidence rate of tuberculosis is growing at approximately 1.1% per year, and the number of new cases—at 2.4% per year. Currently almost one third of the world’s population is infected by the bacterium.\(^{(91)}\) The problem is being aggravated by increasing resistance against the frontline drugs and synergy of this disease with HIV and mycotic infections in immunocompromised patients.\(^{(92)}\) No new drug against tuberculosis has been developed in the last 30 years.\(^{(93)}\) Hence there is an urgent need for new antituberculous agents, preferably having a different mode of action than these presently in use, therefore, the search for new antimycobacterials is the subject of numerous recent studies.\(^{(94-98)}\)
Previous studies on a variety of substituted benzimidazole have shown that 2-substitution combined with halogenation of the benzene ring of the benzimidazole core endows the resulting derivatives with considerable potential for inhibiting growth of diverse microbial and protozoal species.\(^{(99-102)}\) Previous studies also indicated that various nitrobenzylsulphanyl substituent's in position 2 markedly enhanced antimycobacterial activity of benzene ring unsubstituted benzimidazoles\(^{(103)}\); notably, this effect was even more prominent in the 5-methylbenzimidazole series studied.\(^{(104)}\)

1.5. Introduction to antioxidant

The free radical theory of aging stemmed from the study of unstable atoms in living cells and the damage they caused as they tried to stabilize.\(^{(105, 106)}\) Free radicals are highly reactive because of the unpaired electron(s) that seek to be paired but, in turn, damage cells, proteins, lipids, and DNA by altering their structures.\(^{(107)}\) Free radicals happen naturally in the body whenever metallic ions, enzymes, or cellular materials combine with oxygen and are also introduced into the body through toxins, pollutants, and tobacco smoke. Low-level, free radical damage is theorized to accumulate over time, especially mutations in mitochondrial DNA (mtDNA), resulting in aging characteristics.\(^{(107, 108)}\) Most organisms have defense mechanisms to limit the effects of free radicals and to repair the damage left behind, but because not all of the repairs can be fixed, the damage accumulates.\(^{(106, 109)}\) The accumulated mutations of the mtDNA result from a decrease in or loss of function of the natural antioxidant defense layer in the body and cells.

Oxidation commonly involves free radical mechanism. Free radicals are molecules or ions containing unpaired electrons. Reactive oxygen species (ROS) is a term, which encompasses all highly reactive oxygen – containing molecules, including free radicals. Types of ROS include the hydroxyl radical, hypochlorite radical and various lipid peroxides. All are capable of reacting with membrane lipids, proteins and enzymes and other small molecules, resulting in cellular
damage. To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals.

A chemical reaction, that usually takes place at ambient temperature between atmospheric oxygen and organic compound is generally considered as autoxidation. Autoxidation phenomena could be completely prevented by total exclusion of oxygen or other oxidizing substances from biological system. This is generally not practical, but changes in endogenous factors such as addition of inhibitors may decrease the reaction rate or prolong the induction period. Substances that can suppress autoxidation are referred as inhibitors or antioxidants.\(^{(110)}\)
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1.6. **References**

9. BE 626307; CA 60 C. 1964.


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