BENZOTRIAZOLE – THE MOLECULE OF DIVERSE BIOLOGICAL ACTIVITIES

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ABSTRACT

The extensively clinical use of triazole-based medicinal drugs has been promoting increasing effort to develop new structural triazole derivatives. Benzotriazoles as fused aromatic nitrogen heterocycles of benzene ring with triazole exhibit wide potentialities in medicinal chemistry since some anticancer benzotriazole compounds like vorozole and 4,5,6,7-tetabromo-1H-benzotriazole (TBB) were used in clinical therapy. Outstanding developments of benzotriazole compounds in medicinal chemistry, including as anticancer, antifungal, antibacterial, antitubercular, antiviral, antioxidative, antiparasitic, antioxidative agents and so on. Hopefully, this contribution will be helpful to develop benzotriazole-based drugs with high bioactivity and low toxicity.

KEYWORDS: Benzotriazole; Anticancer; Antifungal; Antibacterial; Antitubercular; Antiviral; Antiparasitic; Antioxidative.

INTRODUCTION

Azole heterocyclic compounds exhibit wide range of medicinal applications in the treatment of various types of diseases. [1] Especially, triazole derivatives as medicinal drugs have been playing important roles in medicinal chemistry. [2-4] Have also been found to be widely used in clinic. Benzotriazole is a fused aromatic nitrogen heterocycle of benzene ring with triazole, and its derivatives have been paid increasingly special attention due to their widely potential applications as medicinal drugs, [5-12] corrosion inhibitors, [13] man-made materials, [14] and supramolecular ligands, [15] therefore large numbers of researches have already been focused on this attractive area. Notably, bioactive benzotriazole-based compounds are being deeply exploited all over the world to treat different kinds of puzzling diseases like cancers and the...
current researches of benzotriazole derivatives in medicinal chemistry. Recently, more and more benzotriazole derivatives with effective pharmacological properties, low toxicity, few side effects, little multi-drug resistance, good water solubility, promising bioavailability, diversity of drug administration as well as broad bioactive spectrum have been frequently discovered. Benzotriazole compounds in medicinal chemistry, including as anticancer, antifungal, antibacterial, antitubercular, antiviral activity.[16]

CHEMISTRY
Benzotriazole is a fused aromatic nitrogen heterocycle of benzene ring with triazole, and its derivatives have been paid increasingly special attention due to their widely potential applications as medicinal drugs, corrosion inhibitors, man-made materials, and supramolecular ligands, therefore large numbers of researches have already been focused on this attractive area. Different from triazole, the fused benzene ring makes benzotriazole nucleus possess a larger conjugated sys, resulting in a broad spectrum of biological activities. Benzotriazole moiety has been commonly employed to construct in them to form π-π stacking interactions, and its three nitrogen atoms make it easy to form hydrogen bonds and coordination bonds, thereby benzotriazole derivatives are more ready to bind with a variety of enzymes and receptors in biological system via diverse non-covalent interactions vative drug molecules.[17]

Benzotriazole features two fused rings. Its five-membered ring can exist in tautomers A and B, and the derivatives of both tautomers, structures C and D also can be produced.[18]

Synthesis
a) A synthesis of the BTA involves the reaction of o-phenylenediamine, sodium nitrite and acetic acid. The conversion proceeds via diazotization of one of the amine groups.
The synthesis can be improved when the reaction is carried out at low temperatures (5-10 °C) and briefly irradiated in an ultrasonic bath.\textsuperscript{[19]}

b) N-Alkylation of Benzotriazole under Solvent-Free Conditions:-An efficient, simple and solvent-free method for highly regioselective N-alkylation of benzotriazole in the presence of SiO\textsubscript{2}, K\textsubscript{2}CO\textsubscript{3} and tetrabutylammonium bromide (TBAB) under thermal and microwave conditions has been described. In this method, 1-alkyl benzotriazoles were obtained regioselectively in moderate to high yields and short reaction times. Benzotriazoles are formed by cooling and stirring of benzene-1, 2-diamine with carboxylic acid. Benzotriazole moiety possessing antifungal activity (Compound b had good activity).\textsuperscript{[20]}

\[ R = \text{Aryl, Alkyl} \]
Pharmacological Activities

Anticancer Benzotriazoles

A variety of anticancer drugs such as alkylating agents, platinum complexes, porphyrin drugs and azole agents have been successfully developed and clinically used to treat various cancers. However, most of the clinical anticancer drugs are often toxic to normal tissues, thus causing numerous side effects, which, in turn, limit the treatment efficacy. Long term effectiveness is also limited by dose-related cumulative cardio toxicity as well as drug resistance.\textsuperscript{[21]} Therefore, an increasing number of researches have been directing towards the design and development of new therapeutic agents for the treatment of cancers. Several benzotriazole derivatives have been found to possess potent anticancer activity, for example, the antineoplastic agent vorozole that is in clinical trial, and 4, 5, 6, 7-tetrabromobenzotriazole (TBB) is a commercial available anticancer drug with high selective inhibition against protein kinase CK2. The successful exploration of TBB stimulates the continuous effort towards the development of novel benzotriazole-based anticancer agents targeting various kinases or receptors. The inhibition of kinases is one of the most important pathways to treat cancers attributing to the significant roles of kinases in cell multiplication.\textsuperscript{[22]} The special structure of benzotriazole derivatives could readily bind with different kinases via multiple non-covalent forces such as hydrogen bonds, coordination, ion-dipole, cation-\(\pi\), \(\pi-\pi\) stacking, hydrophobic effect and van der Waals force, thus effectively inhibiting the activity of various kinases including protein kinases CK2 and CHK1, histone deacetylases and focal adhesion kinase and so on. Researches revealed that its four bromine atoms in benzotriazole ring were essential requirement for the inhibitory activity.\textsuperscript{[23]}

\[
\begin{align*}
R_1 &= \text{Br} \\
R_2 &= \text{H} \\
R_1 &= \text{Br} \\
R_2 &= (\text{CH}_2)_3\text{OH} \\
R_1 &= \text{Br} \\
R_2 &= (\text{CH}_2)_3\text{NH}_2
\end{align*}
\]
The efficiency of cancer chemotherapy is usually impaired by drug resistance. Benzotriazole derivative 5 was designed and synthesized to enhance the chemosensitizing activity to combat drug resistance.\textsuperscript{[24]}

![Chemical structure of 5](image)

**Antifungal Benzotriazole**

Fungal infections are a kind of quite prevalent diseases. Among different kinds of antifungal agents,azole compounds have been rapidly developed as the mainstream for fungal infection treatment and are widely used in clinic.\textsuperscript{[25]} Benzotriazole with a benzene ring endures a larger conjugated system than triazole or imidazole as well as a three-nitrogen containing structure could more readily bind with the receptors in organisms with less toxicity. A lot of researches and exploitations have been devoted to benzotriazoles due to their potentiality as novel antifungal agents.\textsuperscript{[26]}

a) **Structural modification of clinical antifungal drugs by benzotriazole ring**

A variety of antifungal azoles representing as an important class of nitrogen-containing heterocycles with desirable electron-rich properties, have been early discovered and successfully used to develop 5 clinical agents. With the growing emergence of the intrinsic and acquired antifungal resistance caused by the abuse of available drugs, especially the multidrug-resistant fungi. Notably, the structural modification of clinical azole antifungal drugs like fluconazole and clotrimazole is regarded as a helpful strategy to improve their physicochemical property and binding affinity, overcome their shortcomings, and effectively broaden their antifungal spectrum.\textsuperscript{[27]} Fluconazole is a first-line oral triazole-antifungal drug recommended by WHO, and could effectively inhibit the growth of *Candida albicans* (*C.albicans*) and *Cryptococcus neoformans* (*C.neoformans*) by displacing lanosterol from cytochrome P45014αDM, blocking the biosynthesis of ergosterol which is the essential component of the fungal cell
membrane, then destroying the integrity of the fungal cell wall and inhibiting the growth and breeding of fungi. However, the treatment efficiency of fluconazole with poor water solubility is limited against some resistant fungal stains like invasive Aspergillosis niger (A. niger). The structural modification of fluconazole is a useful way to explore novel antifungal agents. Recently, some researches have introduced benzotriazole ring into fluconazole to improve its bioactivity. For example, methyl benzotriazole substituted fluconazole derivative 13a showed the increased antifungal activity against Candida glabrata (C. glabrata) with the minimum inhibitory concentration (MIC value) of 25 μg/mL, which was at least two-fold more potent than fluconazole. The introduction of a methyl group into benzotriazole ring of compound 13a at 5-position yielded fluconazole analog 13b with much more superior antifungal activity to fluconazole against A. niger. When the methyl group on benzotriazole was replaced by other groups such as nitro or methoxy group, the inhibition against A. niger considerably reduced, but without significantly affecting their inhibitory activities against Candida spp. The structure activity relationship suggested that small hydrophobic methyl group on the benzotriazole ring made contributions to their inhibitory activity against both Candida and Aspergillus.

![Chemical structure of fluconazole derivatives](image)

13a; R=H  
13b; R=CH₃

b) New structural benzotriazoles as antifungal agents

The combination of multiple functional groups with different action modes into one molecule could produce new antifungal agents. Heterocyclic molecules usually containing N, O or S heteroatom in their cyclic structures as one of the most active classes of compounds possess a wide spectrum of biological activities, and have showed large potentiality in pharmaceutical
science. The introduction of benzotriazole ring into other heterocyclic scaffolds to form some new structural compounds with improved antifungal. The modification by the alkyl or aryl halide could result in good antifungal activities. Aryl halide benzotriazole derivative displaye comparable antifungal efficacy against *Microsporum canis* to the standard drug griseofulvin with an MIC value of 2 μg/mL.\(^{[29]}\)

text continues...
available for clinical use, the treatment of bacterial infectious diseases still remains an important and challenging problem due to a series of factors such as emerging infectious diseases, severely adverse effects, narrow antibacterial spectrum as well as single dosage form.\[50\] More importantly, an increasing number of multidrug resistant microbial pathogens like methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenems-resistant *Enterobacteriaceae* force a real need to develop new compounds acting through distinct mechanisms from the well-known classes of antibacterial agents. The development of benzotriazole derivatives as antibacterial drugs has become a rapidly developing field with considerable breakthroughs.\[31\]

a) **Structural modification of clinical antibacterial drugs by benzotriazole ring**

Structural modification of clinical antibacterial drugs to broaden their antimicrobial spectrum and increase therapeutic indexes has provoked special interest in the realm of medicinal chemistry. Some researchers have manifested that the incorporation of Benzotriazole ring into clinical drugs could evidently improve their antibacterial efficiency and reduce cytotoxicity. Quinolones as essential antibacterial agents are of great importance in clinic. Levofloxacin is one of the third generations of fluoroquinolone antimicrobial drugs, which has been widely used in the treatment of bacterial infections due to its great inhibitory activity against both Gram-positive and Gram-negative bacteria *via* inhibiting DNA gyrase.\[32\]

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\text{NH}_2
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\text{O}_2\text{N}
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\text{O}_2\text{N}
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b) **Benzotriazole-containing metal complexes as antibacterial agents**

Benzotriazole containing ruthenium (III) complex was found to be much more efficient against Gram-negative *Escherichia coli* in comparison to 1,2,3-benzotriazole ligand and precursor ruthenium compounds. The increased lipophilicity of this complex reduced the permeability barriers of the cells and retarded the normal cell process of bacteria, thus resulting
in enhanced antibacterial activity. Additionally, their positive reactivity and inherent bioactivity may throw a new light on the future of benzotriazole-based ruthenium (III) complexes as supramolecular exerted moderate inhibitions against both Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*),[33] which were also much better than that of complexes with other transition metal ions. The variation in structure on coordination of this complex may affect the growth of microorganisms, then resulting in increased bioactivity or reduced toxicology of metal ions towards some organisms.[34]

**Antitubercular Benzotriazole**

*Tuberculosis (TB) is a highly infectious* disease primarily caused by *Mycobacterium tuberculosis*. Several types of antitubercular agents such as isoniazide and rifampicin are available for clinic. However, with the frequent occurrence of resistant strains and clinical adverse drug reactions of stomach and gut as well as liver damage, the uses of clinical anti-TB drugs have been limited by the reduced efficacy and inevitable toxic side effects. Therefore, there is necessary to develop new potent anti-tubercular drugs without cross resistance from known antimycobacterial agents. Recently, more and more researches have shown that the nitrogen heterocyclic benzotriazole compounds have considerable potentiality to treat tuberculosis. The substitution of benzotriazole ring by halogen atoms on the benzene ring has been proved to be a useful way to enhance the bioactivity of benzotriazole derivatives. Sydnones have drawn increasing attention in the fields of both heterocyclic chemistry and medicinal chemistry due to their structural features and biological activities. Some amide benzotriazole derivatives synthesized from sydnone fragment were reported to display good antitubercular activities. For instance, amino benzotriazole was manifested to be a potent antitubercular agent with better inhibition against *M. tuberculosis* than standard drugs streptomycin and pyrazinamide.[35] Pyrazole N-aryl derivatives have been deeply investigated in the pharmaceutical field due to their wide range of bioactivities such as anti-hyperglycemic, analgesic, anti-inflammatory, antipyretic and antibacterial activities. The introduction of pyrazole ring in molecules could increase the electron density of the system and makes the chromophore more resistant towards enzymatic reduction by radical species.[35]

**Antiviral Benzotriazoles**

Virus is a class of infinitesimal pathogen. Viral infections cause about 60% of epidemic infectious diseases and seriously threaten to human health. Traditional nucleosides are promi-
nent drugs used to treat viral infections. However, the structural modifications of nucleosides are faced with a major challenge because of poor solubility in common organic solvents.[36] Moreover, the current antiviral agents can not only inhibit the growth of virus instead of directly destroying and killing them, but also damage the host cell. For these reasons, large numbers of investigations have been focused on the design and development of non-nucleoside compounds as novel antiviral drugs in recent decades. The exploitation of new antiviral benzotriazole compounds has opened a new opportunity in this field. Hepatitis C is a liver disease caused by the hepatitis C virus (HCV). HCV is a single-stranded positive RNA virus in the family of flaviviridae, which is associated with severe liver diseases including cirrhosis, liver cancer, and liver failure, and large numbers of people worldwide are chronically infected by this virus. Some novel aryl thiourea derivatives have been found to possess potent activity with nanomolar range in a cell-based HCV replicon assay. However, these compounds have significant cytotoxicity and poor pharmacokinetic activities. The newly synthesized thiourea benzotriazole derivative 50 could inhibit HCV subgenomic replication with a moderate efficiency.[36] Importantly, this compound showed lower cytotoxicity and better pharmacokinetic activities than previously synthesized aryl thiourea derivatives. Preliminary SAR study of this compound is currently under active investigation, found to have antiviral abilities. For example, compound 51 exhibited a significant antiviral effect on Respiratory Syncytial Virus (RSV) with an EC50 value of 0.1 μg/mL, which was more effective than the reference drug azauridine.[37] The presence of benzotriazole might reduce the cytotoxicity and contribute to its high selectivity index. The persistent infection with hepatitis B virus (HBV) remains a seriously global healthy problem. However, the clinical available nucleoside analogues can lead to low response rate in the patient and result in the development of drug-resistant virus after long term treatment, so this condition promotes to explore novel non-nucleoside antiviral drugs with excellent inhibitory activity on the replication of HBV DNA and the secretion of HBV e antigen (HBeAg) and HBV surface antigen (HBsAg). The development of new anti-HBV agents is focused on discovering diverse compounds with either novel structures or a new mechanism of action. The new structural benzotriazole derivative displayed significant ability in reducing the secretion of HBsAg and HBeAg with better IC50 values of 33.7 and 111.4 μg/mL, respectively, than clinic antiviral drug tenofovir.[37]

**Antiparasitic Benzotriazole**

Parasitosis is a kind of epidemic diseases causing serious damages to both society and economy. This disease is associated with infectious parasite present multiformity, including hel-
minthiasis and protozoiasis etc. Several benzotriazole derivatives with advanced antiparasitic activity have showed potentiality to solve this problem. Amebiasis, caused by the protozoan parasite *Entamoeba histolytica* (*E. histolytica*), is responsible for large numbers of deaths and many people infected with *E. histolytica*. However, there are still 90% of patients remain asymptomatic while carrying the infection for several years, estimated by WHO. The clinical drugs such as azomycin are faced with parasite resistance and negative side effects. In order to investigate the novel compounds with effective, benzotriazole derivative was obtained and possessed low micromolar activity, which was more active than metronidazole, the clinical choice for the treatment of amebiosis.\(^{[38]}\) When the chlorine atom in compound was changed into methyl group, the antiprotozoan activity greatly decreased (IC\(_{50}=3.248\, \mu g/mL\)). These results indicate that the benzotriazole scaffold represents an excellent starting point for an optimization of novel antiparasite drugs.

The newly synthesized N-benzenesulfonyl benzotriazole showed good inhibitory activity against epimastigotes of *Trypanosoma cruzi*, whereas the standard benzotriazole exhibited no inhibitory on the growth of this parasite form. These results revealed the potentiality of compound 56 as a prototype in drug design for developing new anti-*T. cruzi* agents.\(^{[39]}\)

**Antioxidative Benzotriazoles**

Free radicals, represented by reactive oxygen nitrogen species from human metabolism, could produce harmful substances by a variety of metabolic pathways, then cause healthy problems, such as aging, cancer and many neurodegenerative diseases. Therefore, eliminating the excessive oxidized free radicals, improving the antioxidative activities of the body to resolve the aging-related diseases has been an increasingly important challenge. Antioxidants are reducing agents used to stabilize some free radicals produced by cellular metabolism.\(^{[40]}\) Benzotriazole compounds have shown remarkable antioxidative activities and large potentiality to be novel antioxidative agents or candidates. Primaquine (PQ) derivatives are well-known and wide-used antimalarial drugs, meanwhile they are interesting molecules to develop potential antioxidative agents due to their prooxidant effects in blood. Benzotriazole substituted primaquine 58 showed a higher interaction (73.8%) than the parent compound primaquine (31%), and it also exhibited a good lipoxygenase inhibitory (LOX) inhibition.\(^{[41]}\) In addition, benzotriazole derivative had perfect DPPH interaction value (85%), which was comparable to that of the reference compound nordihydroguaiaretic acid (91%) at the same concentration. This compound also displayed a good lipid peroxidation (LP) inhibition of 31%. These results proved the promising efficiency of the benzotriazole group as a new scaffold in
the rational design of new antioxidant compounds. Ketoprofen (Ket) is a non-steroidal anti-inflammatory drug (NSAID) with pronounced analgesic and antipyretic activities. Recently, the structural modification of ketoprofen molecule has afforded a series of derivatives with minimized side-effects, prolonged plasma half life, increased solubility and considerable antioxidative potentiality. For example, ketoprofen benzotriazole derivative possessed good interaction with 1, 1-diphenyl 1-2-picyrylhydrazyl (DPPH) which was a stable free radical with spared electron delocalization over the whole molecule. The interaction between compound and DPPH indicated its radical scavenging ability in an iron-free system as well as its reducing activity. Moreover, it was proved to be an excellent inhibitor of LP of 98%, which was significantly higher than that of the standard ketoprofen (69.3%). This compound also exhibited remarkable soybean LOX activity of 95%.[42] The replacement of benzotriazole ring by other substituent such as pyrrolyl or piperidyl fragment could obviously reduce the antioxidant activity, which indicated that then presence of benzotriazole was benificial to its antioxidant propriety.[42]

Benzotriazole as Other Medicinal Agents
Apart from the above mentions, benzotriazole compounds also exhibited potential applications in other medical fields, including as anti-inflammatory, antidiabetic agents, antimalarial agents and so on. Inflammation is a complicated disease with a series of uncomfortable symptoms caused by tissue injury, infection of trauma or biochemical stimulation. Pain is one of the classic signs of the inflammatory process induced by different chemical mediators released during this process leading to nociceptive sensitization. A lot of nonsteroidal anti-inflammatory drugs (NSAIDs) are available for the treatment of pain and inflammation. However, most of NSAIDs show limited antiinflammatory efficacy and cause various side effects such as gastrointestinal ulcers and hemorrhages. Therefore, much effort dedicates to exploit novel and effective NSAIDs. Recently, more and more literature indicated that benzotriazole derivatives have potentiality in the treatment of inflammations.[43] Cytosolic phospholipase A2α (cPLA2α) is an attractive target for the design of new anti-inflammatory drugs, since the inhibition of cPLA2α could lead to the blockade of cellular production of all these inflammatory lipid mediators. Benzotriazole-6-carboxylic acid 61 displayed good inhibition of cPLA2α. The replacement of carboxyl benzotriazole into carboxyl indole ring or carboxyl benzimidazole resulted in decreased inhibitory activities.[44] These results suggested that the benzotriazole ring played an important role in enhancing its antiinflammatory ability.[44]
CONCLUSION

As can be seen from the above mentions, benzotriazole-based compounds with various outstanding bioactivities have become increasingly active in the field of medicinal chemistry. Importantly, some anticancer benzotriazole compounds such as vorozole and TBB have been clinically used. Currently, the researches and developments of benzotriazole compounds have been focused on the following two main aspects.

On the one hand, an increasing effort is the structural modification by the introduction of benzotriazole ring into available drugs, and focused more on new strucutral benzotriazole-containing compounds with novel mechanisms of action. The electron-rich benzotriazole ring with a large conjugated system is an attracting molecular skeleton, which is not only easily modified by various types of functional groups, but also employed to combine with other bioactive fragments to afford more active compounds with remarkable physicochemical properties.

On the other hand, the design and developments of benzotriazole-containing metal complexes will become an actively important direction. Three-nitrogen containing conjugated Benzotriazole compounds with promising charge-transfer property are ready to form hydrogen bonds as well as cooperation bonds. Undoubtedly, with increasing effort directly towards bioactive benzotriazole compounds, a growing number of Benzotriazole derivatives will inevitably be used in clinic and make remarkable contributions to human’s health.

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