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# Novel purine benzimidazoles as antimicrobial agents by regulating ROS generation and targeting clinically resistant *Staphylococcus aureus* DNA groove

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ABSTRACT

A novel series of purine benzimidazole hybrids were designed and synthesized for the first time with the aim to circumvent the increasing antibiotic resistance. Hexyl appended hybrid **3c** gave potent activities against most of the tested bacteria and fungi especially against multidrug-resistant strains Staphylococcus aureus (MIC = 4 μg/mL). Structure-activity relationships revealed that the benzimidazole fragment at the 9-position of purine played an important role in exerting potentially antibacterial activity. Both cell toxicity and ROS generation assays indicated that the purine derivative **3c** showed low cytotoxicity and could be used as a safe agent. Molecular modeling suggested that hybrid **3c** could bind with the residues of Topo IA through hydrogen bonds and electrostatic interactions. Quantum chemical studies were also performed on the target compound **3c** to understand the structural features essential for activity. The active molecule **3c** could effectively interact with S. aureus DNA to form **3c**–DNA complex through groove binding mode, which might block DNA replication to display their powerful antimicrobial activity.



Microbial infection is a common and frequently occurring infectious disease worldwide. A large number of antibiotics and synthetic drugs have been widely used in clinic and have played an important role in the treatment of microbial infections.[1] However, the high morbidity and mortality caused by bacteria and fungi are still increasing serious threat due to the appearance of drug-resistant strains in recent decades.[2] Multiple strategies have been adopted to prevent and control antimicrobial resistance, one useful strategy is to develop the new drug hybrids which are generally considered a practical promise to tackle the growing problem of drug resistance.[3]

Purines can be regarded as one of the most ubiquitous and functional *N*-heterocyclic compounds found in nature. Purine scaffold is the core structural fragment of adenine and guanine in RNA and DNA.[4] Purine nucleotides such as ATP, GTP, cAMP, cGMP, NAD and FAD act as co-factors, substrates or mediators in the function of many proteins.[5] These proteins are estimated to include half of the most pharmaceutically available targets, primarily enzymes and receptors. Some naturally existing and synthetic purines have been applied as antitumor, antiviral and antiparasitic agents and adenosine receptor ligands while the application in antimicrobial aspect has rarely been reported.[6] Therefore, the development of purine-based novel antimicrobial agents is of significant value in medicinal chemistry. In view of the pivotal role of purines in the regulation of many biological processes, it arouses our immense interest to modify the purine skeleton using isosteric ring systems to develop novel structural molecules and investigate their possibility as new antimicrobial agents.

Azoles as nitrogen-containing five-membered aromatic heterocycles with desirable electron rich characteristic can readily bind with a variety of enzymes and receptors in biological systems through diverse weak noncovalent interactions, thereby exhibiting broad bioactivities.[7] Thus, the combination of azoles and the purine nucleus provides a great prospect for medicinal and biological application. They have been developed as antimycobacterial and antiviral drugs and also as adenosine receptor agonists and antagonists. Especially, benzimidazole with special benzene-fused imidazole ring as a key component for various biological activities like antiparasitic, anticancer, anti-inflammatory and antiulcer agents etc. has attracted considerable concern.[8] Recently, extensive

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biochemical and pharmacological studies revealed that benzimidazoles possessed large potentiality to inhibit the growth of bacterial and fungal strains.[9] Further research disclosed that benzimidazole nucleus structurally resembling with purine scaffold in nucleotides was sometimes addressed as 1,3-dideaza-purine and had the capability of competing with purines, distinctly inhibiting the synthesis of nucleic acids and proteins, thereby killing bacterial strains or inhibiting their growth.[10,11] Clearly, benzimidazole-based derivatives possess great potential as new antibacterial and antifungal agents. Reasonably, this structural similarity prompts us for the first time to combine benzimidazole moiety and purine backbone to generate a novel type of hybrids as potentially antimicrobial agents (Fig. 1) and evaluate their antimicrobial potency.

Design idea for target compounds was mainly from three aspects: (I) Structural modification on the benzimidazole nucleus; (II) Structural change on the purine scaffold; (III) The different linkers between benzimidazole and purine rings. The related considerations were given as follows:

(1) The C-6 and N-9 positions in the purine scaffold are the most widely exploited anchoring points and the substituted purines are commercially available.[12] Therefore the purine nucleus through the N-9 position with benzimidazoles was hybridized;
(2) The incorporated flexible $-CH_2-$ aliphatic linker between benzimidazole and purine nucleus is helpful for regulating the molecular conformation to interact with targets. The *N*-alkyl benzimidazole moiety was reported to have good performance in exerting bioactivities, the$-CH_2CH_2-$ aliphatic linker was also introduced into target compounds in order to evaluate its contribution towards antimicrobial efficacy;
(3) A lot of work revealed that substituents at *N*-containing heterocycle could significantly influence the pharmacological properties by adjusting lipid-water partition coefficient and binding affinity. Rationally, various aliphatic chains with different lengths and substituted phenyl moieties including chloro and fluoro groups were introduced at N-1 position on benzimidazole moiety to investigate their effect on bioactivities;[13]
(4) The substituted groups at C-2, 5, 6 positions on benzimidazole nucleus had the capacity to modulate the pharmacokinetic properties and enhance the antimicrobial efficacy. The substituents on benzimidazole nucleus were changed to explore their impacts on biological activities;
(5) It is well known that saturated nitrogen heterocycles such as morpholine, piperidine and pyrrolidine are beneficial building blocks in drug design and prevalently present in many approved drugs.[14] The above mentioned alicyclic amines were introduced to C-6 position on purine nucleus for investigating the influence of the substituents in the purine nucleus on structure–activity relationship and bioactive profiles.

The designed structures of this series of novel purine benzimidazoles are shown in Schemes 1 and 2. All the newly synthesized compounds were characterized by spectral analyses and evaluated for their antimicrobial activities *in vitro* against five Gram-positive bacteria, six Gram-negative bacteria and five fungi according to the Clinical and Laboratory Standards Institute (CLSI).[15] Structure-activity relationship, cytotoxicity, reactive oxygen species, molecular docking and quantum chemical studies as well as the preliminary antimicrobial mechanism with DNA to highly active compound were also discussed and investigated.

The target purine benzimidazole hybrids were prepared starting from commercial 6-chloro-9H-purine. Their synthetic routes were outlined in Schemes 1 and 2 and Schemes S1–S2. The reaction of 6-chloro-9H-purine with morpholine at 80 °C produced 4-(9H-purin-6-yl)morpholine **1** and then was further treated by benzimidazoles **8**, **10** and **12** in dimethylformamide to give the derivatives **2**, **3** and **4** respectively in yields of 48.8–71.5% (Scheme 1). The commercial *o*-phenylenediamines were reacted with various carboxylic acids through the cyclization to form benzimidazoles **7a–f**, which were subsequently proceeded *N*-alkylation with 1,2-dibromoethane in acetonitrile using potassium carbonate at 70 °C to afford *N*-substituted benzimidazoles **8a–f** in 46.3%–54.7% yields (Scheme S1). Intermediates **10** and **12** were prepared from the cyclization of *N*-mono substituted *o*-phenylenediamine with chloroacetic acid in hydrochloric acid at reflux in 74.2–83.6% yields. It was noticed that *N*-monosubstituted *o*-phenylenediamines **9** and **11** were provided in yields of 62.4–69.7% by the nucleophilic substitution reaction of *o*-phenylenediamine with a series of halogenated aliphatic chains at ratio of 1.2: 1 (Scheme S2). It was reported that the presence of aliphatic nitrogen heterocycles had some influence on the bioactivity, and thus target compounds **6a** and **6b** were further prepared to explore their effect on the bioactivities (Scheme 2). The starting material 6-chloro-9H-purine was also reacted with piperidine and pyrrolidine in ethanol at reflux using triethylamine as base to produce intermediates **5a** and **5b** with 92.7% and 91.4% yields, respectively. Further coupling with *N*-hexyl benzimidazole **10c** in dimethylformamide at 50 °C was carried out to generate the final products **6a** and **6b** in 67.4% and 69.3% yields.

The *in vitro* antimicrobial activities for all the target compounds were evaluated for five Gram-positive bacteria (Methicillin-Resistant *Staphylococcus aureus* N315 (MRSA), *Enterococcus faecalis*,

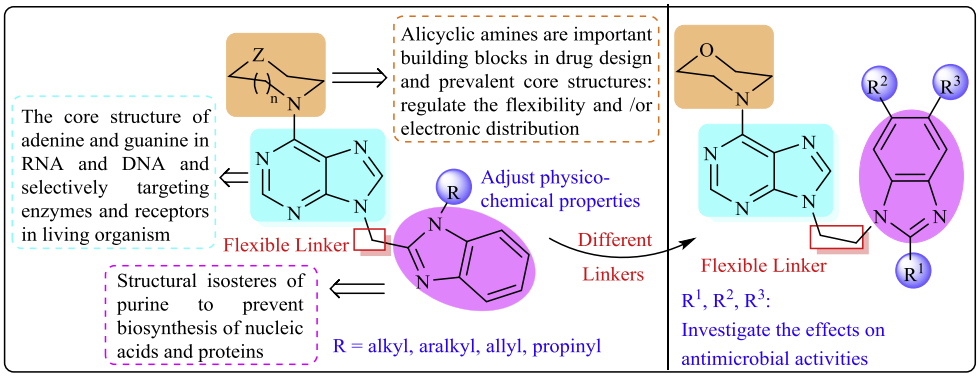


**Fig. 1.** Design of novel purine benzimidazole hybrids.