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# Accepted Article

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# Design, microwave-assisted synthesis and in vitro antibacterial and antifungal activity of 2,5-disubstituted benzimidazole

Yanpeng Shi[a], Kai Jiang[b], Ran Zheng[a], Jiaxu Fu[a], Liuqing Yan[a], Qiang Gu[a], Yumin Zhang*[a], Feng Lin*[b]

[a]*College of Chemistry, Jilin University, Changchun 130012, P. R. China*

[b] *School of life Sciences, Jilin University, Changchun 130012, P. R. China*

*Corresponding Author: Professor Yumin Zhang and Professor Feng Lin

Tel: (+86)-431-85168470 (3)

Fax: (+86)-431-85168441

E-mail: zhang_ym@jlu.edu.cn

**Abstract** Seventeen novel 2,5-disubstituted benzimidazole derivatives were designed, synthesized and evaluated for their antibacterial activities. The tested compounds **B1**–**B4** and **C2**–**C6** exhibited not only good antifungal activity but also favorable broad-spectrum antibacterial activity. Also, the lowest MIC of antibacterial and antifungal activity was 2 μg/mL and 4 μg/mL, respectively. It suggested that the structure of compound including the different substituent and its sites directly affected on efficacy of the synthesized compound.

Keyword: 2,5-disubstituted benzimidazole, microwave-assisted synthesis, antimicrobial activity

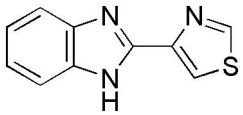
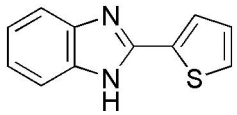
## Introduction

Since the early 1900s, the incidence of systemic bacterial and fungal infections has been increased in relatively high rates of morbidity and mortality.[1–3] This is because of an enhanced number of immunocompromised patients who are impressionable to these infections. Several research teams have indicated Candida albicans (*C. albicans*) is the most common fungal species in these patients.[3–5] *C. albicans* is a commensal fungus resides in the skin and the human gastrointestinal. It can cause serious mucosal infections along with fatal invasive infections in potential individuals, who subjected to immunodeficiency result of chemotherapy for cancer, immune suppression for the transplants of stem cells or solid organs, or HIV infections.[6–8] Recently, although many new drugs have been used in clinical treatment, *C. albicans* sustained to dominate as the predominant causative agent of systemic fungal infections.[9]

Additionally, it is well-known that the bacterium causing human diseases are mainly gram-negative and positive bacteria all the time. [10][11] For example, *Pseudomonas aeruginosa* (*P.*

*aeruginosa*) is one of the gram-negative opportunistic pathogens, which can extensively causes all kind of clinical infections such as people getting severe burnt, AIDS, diabetes patients and cystic fibrosis(CF) sufferers.[12][13] *P. aeruginosa* is one of the most epidemic and clinically significant pathogens for CF of sufferers. All gram-positive bacteria infections were attributed to multi-drug-resistant gram-positive bacteria expression a major public health professions problem in the world, and are always related with high mortality rates.[14] It is estimated that *Staphylococcus aureus* (*S. aureus*) as a gram-positive bacteria is in total found about 20–30% of the human population for nasal passages, skin and mucous membranes.[15][16] *S. aureus* can lead to the infection of the bloodstream, reduce respiratory track, destroy skin and soft tissue, further result in bacteremia, ventilator associated pneumonia, as well as endocarditis and osteomyelitis illness.[17,18] Besides, antimicrobial resistance, one of the all over worldwide threats, is a fearful matter under review by the world health organization (WHO) and many countries in the globe.[19–22] *Staphylococcus epidermidis* (*S. epidermidis*), one of the greatest important gram-positive bacterium, which is among important compromise for the resistant strains[23–25], simultaneously is deemed to a significant nosocomial pathogen influencing low immunity, such as athroplasty, central venous catheters, and analogues. Currently, *S. epidermidis* has become a main clinical medicine problem, and led to vital morbidity and mortality in both people biota group and those with potential complication.[23–25] The resistant strains make complicated to the therapy, and significantly affect the get well of patients.[26] Additionally, fungal resistance also increased. Therefore, it is imperative to design and synthesize new antibacterial and antifungal agents.

It is reported that benzimidazole analogues have good biological and pharmaceutical activities and drug-resistant properties[27][28] including antifungal, antimicrobial, anticancer, etc. because they have a similar structure with a purine which play pivotal role in the synthesis of protein and nucleic acid in the bacteria.[29] 2-Substituted benzimidazole derivatives are reported to exhibit good antimicrobial activity,[30–33] for example, the 2-position of benzimidazole derivatives bearing heterocyclic rings, such as thiabendazole, 2-(thiophen-2-yl)-1*H*-benzimidazole(Fig. 1) and naphthalene ring.[34–36] Especially, thiabendazole without teratogenic, carcinogenic, and mutagenic effects has been manufactured.[29][37] Also, it is reported that benzene ring of benzimidazole with an inductive electron-withdrawing group can improve antibacterial activity.[38][39]

Thiabendazole 2-(Thiophen-2-yl)-1*H*-benzimidazole

Fig. **1** The structure of Thiabendazole and 2-(Thiophen-2-yl)-1*H*-benzimidazole

Moreover, microwave irradiation technology is a rising organic synthesis technology in recent two decades. It had attracted much attention because of its high yield, low waste, energy efficiency, short reaction time and simple operation. With all this in mind and in view of the continuation of our work,[40][41] 2,5-disubstituted benzimidazole compounds including heterocyclic ring or naphthalene ring and electron-withdrawing group were designed and synthesized under microwave irradiation. The synthesized results and the evaluation of antifungal activity against *C. albican*s(ATCC 1023), *P. aeruginosa*(ATCC 27853), *E. coli*(ATCC 25922), *Staphylococcus aureus*(*S. aureus* ATCC 25923, *S. aureus* ATCC 3933) and *S. epidermidis*(ATCC 12228) wish to be reported here.

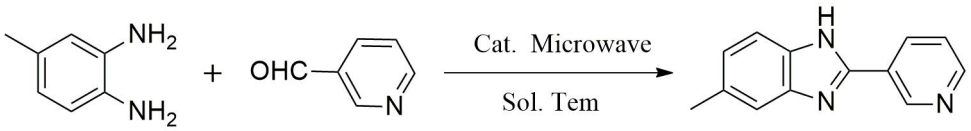
## Results and discussion

### Design, synthesis and reaction conditions' optimization of 2,5-disubstituted benzimidazoles

According to the literature some 2-substituted benzimidazoles were synthesized using $Na_2S_2O_5$ as catalyst and oxidant starting from mono-substituted phenylenediamine under traditional heating and water-ethanol solvent conditions. The method for synthesizing 2-substituted benzimidazoles required not only long reaction time (12 h) but also a plenty of mixture solvent.[42] At the beginning of the study, 6-methyl-2-(3-pyridyl)-1*H*-benzimidazole(**A1**) was designed and synthesized by transforming traditional heating into microwave irradiation. Unfortunately, the desired prodAlso, it is reported that the reaction time was evidently shortened when the reaction was carried out in the presence of $Na_2S_2O_5$ catalyst under solvent-free and microwave radiation condition.[43]uct was not entirely obtained and always accompanied by byproduct *bis*-Schiff bases that was hard to be separated completely, when the reaction started from 4-methylbenzene-1,2-diamine(**a**) and 3-nicotinaldehyde(**D**) using $Al_2O_3$, $Na_2S_2O_5$ or $NaHSO_3$ as catalysts and oxidants and EtOH as a solvent at 78 °C. Also, the yields of the obtained product were respectively 46.2%( $Al_2O_3$, Table 1, entry 3), 68.6%( $Na_2S_2O_5$, Table 1, entry 4) and 54.7% ($NaHSO_3$, Table 1, entry 5). However, no product was obtained using $FeCl_3{\cdot}6H_2O$ and $H_3[PW_{12}O_{40}]$ as catalysts and oxidants. The main reason lied in that $Na_2S_2O_5$ could provide not only an acidic environment for the ring-closure, but also

appropriate ability for in situ oxidizing 2,3-dihydrobenzimidazole to benzimidazole. Further, the catalyst $Na_2S_2O_5$ loading was explored in EtOH solvent (Table 1, entries 4, 6–9). It was observed that the yields of **A1** were gradually increased to 68.6% (1 mmol), followed sequentially decrease to 68.1% (1.25 mmol) and 68.0% (1.50 mmol), along with the reaction times was gradually shortened by increasing $Na_2S_2O_5$ loading from 0.50 mmol to 1.50 mmol at 78 °C. It is suspected that the low yield was related to the low boiling point of ethanol and the solubility of reactants. Besides, the reactants molar ratio (**a**:**D**) were investigated(Table **1**, entries 4, 10–12). The highest yield (74.4%, Table **1**, entry 11) was obtained when the reactants molar ratio **a**:**D** was 1:1.1. Subsequently, four other solvent applied in the reaction and the obtained yields in different condition are shown in Table **1** (entries 13–16). The yield used DMF (83.5%) as a solvent was highest, followed by EtOH (74.4%), and then acetonitrile (47.3%) and ethyl acetate (46.5%). The target product **A1** was not obtained using THF as a solvent. These maybe were related to the solubility of reactants and the tangent value of the solvent loss angle.[44] Because the above reaction that obtained target product was always accompanied by *byproduct bis*-Schiff bases. In order to enhance the yield and purity, the reaction temperatures were increased from 100 °C to 140 °C (Table **1**, entries 16–20). Exciting, not only the by-product was disappeared, but the reaction time was gradually shortened from 15 min to 8 min. The highest yield was up to 92.4 % at 130 °C for 10 min. The possible reason was that the increase of reaction temperature promoted the effective collision of reactants molecule and the ring closure reaction. Therefore, taking into account the perspective of economic cost and environmental protection, the optimized reaction condition was considered as follows: Catalyst select $Na_2S_2O_5$ and catalyst loaded 1.0 equivalent mol of **a**, The molar ratio of material was 1:1.1, DMF as a solvent and microwave irradiation temperature 130 °C (Table 1, entry 19).

**Table 1** Some effect factors of the synthetic compound **A1**




**a** **D** **A1**

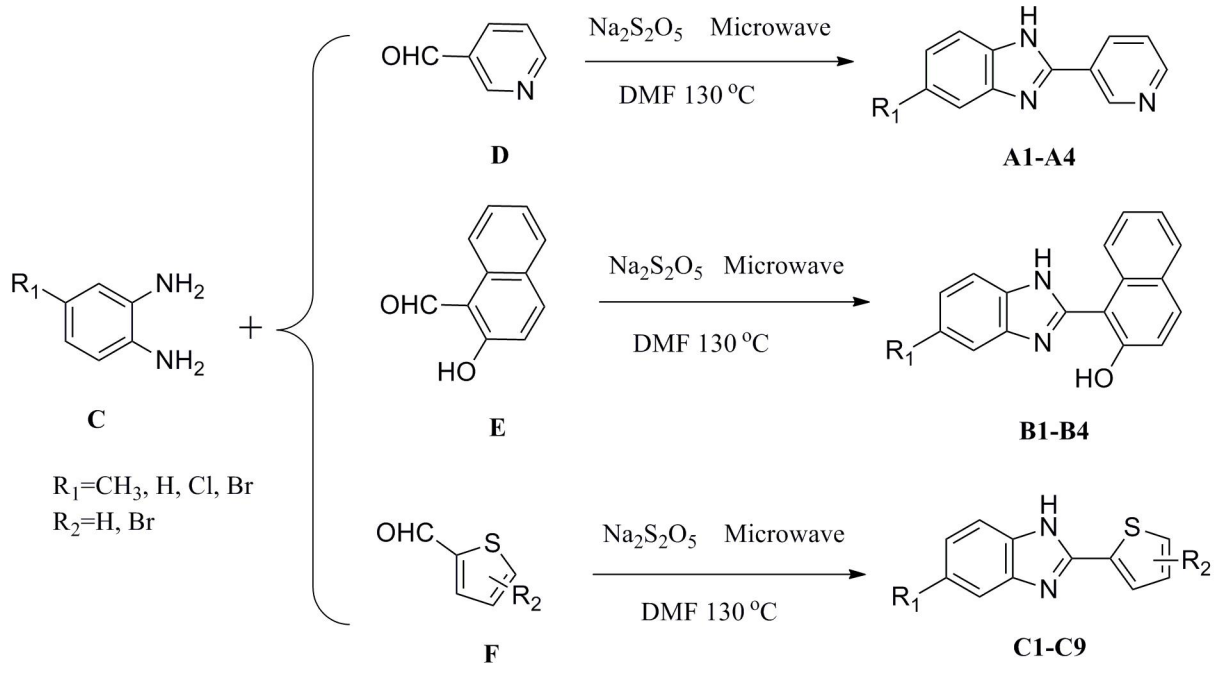
| Entry | Reactant a:D | Catalyst | | Solvent | T/ °C | Time/ min | Yield[a] / % |
|---|---|---|---|---|---|---|---|
| | | Catalyst | equiv | | | | |
| 1 | 1.0:1.2 | $H_3[PW_{12}O_{40}]$ | 1 | Ethanol | 78 | 20 | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2 | 1.0:1.2 | $FeCl_3·6H_2O$ | 1 | Ethanol | 78 | 25 | - |
| 3 | 1.0:1.2 | $Al_2O_3$ | 1 | Ethanol | 78 | 18 | 46.2 |
| 4 | 1.0:1.2 | $Na_2S_2O_5$ | 1 | Ethanol | 78 | 15 | 68.6 |
| 5 | 1.0:1.2 | $NaHSO_3$ | 1 | Ethanol | 78 | 22 | 54.7 |
| 6 | 1.0:1.2 | $Na_2S_2O_5$ | 0.5 | Ethanol | 78 | 18 | 59.2 |
| 7 | 1.0:1.2 | $Na_2S_2O_5$ | 0.75 | Ethanol | 78 | 15 | 63.4 |
| 8 | 1.0:1.2 | $Na_2S_2O_5$ | 1.25 | Ethanol | 78 | 13 | 68.1 |
| 9 | 1.0:1.2 | $Na_2S_2O_5$ | 1.5 | Ethanol | 78 | 13 | 68.0 |
| 10 | 1.0:1.0 | $Na_2S_2O_5$ | 1 | Ethanol | 78 | 13 | 66.8 |
| 11 | 1.0:1.1 | $Na_2S_2O_5$ | 1 | Ethanol | 78 | 13 | 74.4 |
| 12 | 1.0:1.3 | $Na_2S_2O_5$ | 1 | Ethanol | 78 | 13 | 67.6 |
| 13 | 1.0:1.1 | $Na_2S_2O_5$ | 1 | THF | 68 | 25 | - |
| 14 | 1.0:1.1 | $Na_2S_2O_5$ | 1 | $CH_3CN$ | 82 | 25 | 47.3 |
| 15 | 1.0:1.1 | $Na_2S_2O_5$ | 1 | EtOAc | 98 | 20 | 45.6 |
| 16 | 1.0:1.1 | $Na_2S_2O_5$ | 1 | DMF | 100 | 15 | 83.5 |
| 17 | 1.0:1.1 | $Na_2S_2O_5$ | 1 | DMF | 110 | 15 | 85.3 |
| 18 | 1.0:1.1 | $Na_2S_2O_5$ | 1 | DMF | 120 | 12 | 87.9 |
| 19 | 1.0:1.1 | $Na_2S_2O_5$ | 1 | DMF | 130 | 10 | 92.5 |
| 20 | 1.0:1.1 | $Na_2S_2O_5$ | 1 | DMF | 140 | 8 | 91.4 |

*a*: isolated product yield; "-": no product was obtained.

Subsequently, having been optimized the reaction conditions for the model system (Table **1**, entry 19) was chosen to synthesize the designed compounds(Table **2**). A sequence of novel 2,5-disubstituted benzimidazoles including heterocyclic and naphthyl ring had been synthesized in 50.2%–92.5% yields. Seventeen different 2,5-disubstituted benzimidazoles(**A1**–**A4**, **B1**–**B4** and **C1**–**C9**) have been synthesized using $Na_2S_2O_5$ as a catalyst and oxidant under microwave irradiation.(Scheme 1). Various 4-substituted phenylenediamine with 3-pyridylaldehyde (to

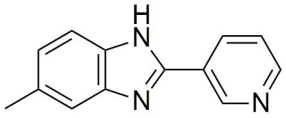
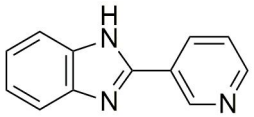
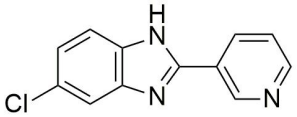
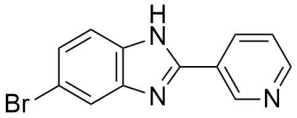
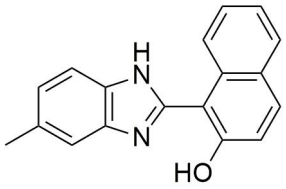
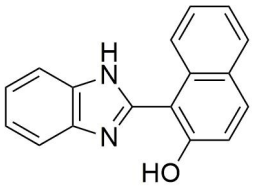
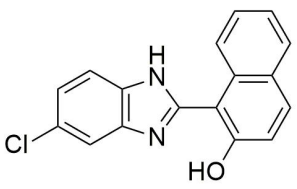
Scheme **1** The route for synthesizing 2,5-disubstituted-benzimidazole.

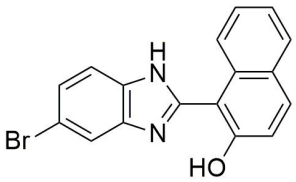
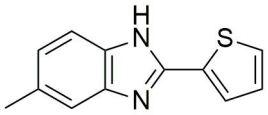
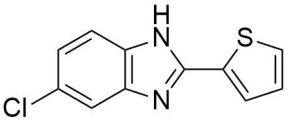
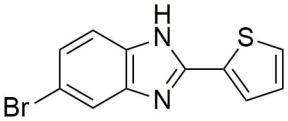
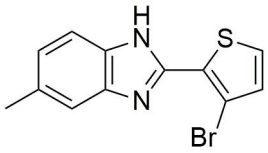
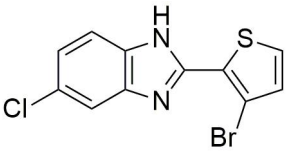
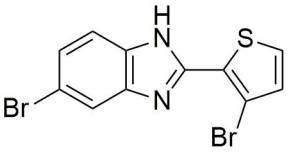
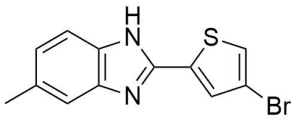
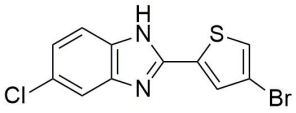
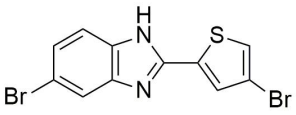



synthesize **A** series products) or 2-hydroxyl-1-naphthaldehyde (to synthesize **B** series products) or various 2-thiophenecarboxaldehyde (to synthesize **C** series products) were carried out by dissolving both reactants and $Na_2S_2O_5$ in DMF in 1:1.1:1 molar ratio and heating them in microwave reactor at 130 °C for 10–15 min. Structure, irradiation time, yield and Mp. of the synthesized products are listed in Table **2**. In this reaction, a nucleophilic amino group of 4-substituted phenylenediamine firstly attacked carbon on aldehyde group of the other reactant giving rise to formation of a single Schiff-base which then further underwent cyclization to afford the target products[45]. It is seen from Table **2** that the yield of the synthesized 2,5-disubstituted benzimidazoles were 55.5–92.5% (Table **2**, entries **A1–A4**), 75.7–89.5% ( Table **2**, entries **B1–B4**) and 50.2–92.1% (Table **2**, entries **C1–C9**), respectively. When 2-substituted group of the obtain 2,5-disubstituted benzimidazoles was the same, the yield of the obtained target product was decreased with the electron-donating ability of 5-substituted group(–$CH_3$, –H, –Cl and –Br) on *o*-phenylenediamines decreasing (Table **2**, entries **A1–A4**). The reason maybe lied in the fact that the electron-donating groups increased the electron cloud density of amino N on substituted *o*-phenylenediamine. It is helpful that the nucleophilic amino group of 4-substituted phenylenediamine attacks carbon on carbonyl group of the corresponding aldehyde and promoting nucleophilic addition reaction. When nicotinaldehyde was as a reactant material, the yield was much higher than that of thiophene-2-carbaldehyde as a reactant (Table 2, entries **A1**, **C1** or **A2**, **C2** or **A3**, **C3** and so all), because electron-poor π feature of pyridine

ring with higher conjugation energy enhanced nucleophilicity of carbonyl carbon. Meanwhile, when 2-hydroxy-1-naphthaldehyde as a reactant, the yield was slightly lower than that of thiophene-2-carbaldehyde as a reactant, but slightly higher with nicotinaldehyde as a reactant (Table 2, entries **B1**, **C1**, **A1** or **B3**, **C2**, **A3** and so all). Additionally, it is also seen from Table **2** that the reaction time shortened with the enhancement of the electron-donating ability of substituted *o*-phenylenediamine. It is not difficult to find that the method proposed in this paper has good applicability by combining the synthesis of the above benzimidazole derivatives and the effect of different substituents on the yield of the target product.

Table **2** The synthesized compounds **A1**–**A4**, **B1**–**B4** and **C1**–**C9**

| **Compound** | **Product** | **Reaction Time** (min) | **Yield** [a] (%) | **Mp.** (°C) |
|---|---|---|---|---|
| A1 |  | 10 | 92.5 | 218-220 |
| A2 |  | 10 | 80.6 | 218-220 |
| A3 |  | 13 | 70.9 | 244-246 |
| A4 |  | 15 | 55.5 | 238-240 |
| B1 |  | 13 | 89.5 | 230-232 |
| B2 |  | 13 | 85.1 | 224-225 |
| B3 |  | 15 | 85.1 | 234-236 |

| | | | | |
|---|---|---|---|---|
| B4 |  | 15 | 75.7 | 130-132 |
| C1 |  | 10 | 90.6 | 238-240 |
| C2 |  | 12 | 67.8 | 226-228 |
| C3 |  | 15 | 51.4 | 196-198 |
| C4 |  | 10 | 85.3 | 214-216 |
| C5 |  | 12 | 69.7 | 216-218 |
| C6 |  | 15 | 50.2 | 261-264 |
| C7 |  | 10 | 92.1 | 218-220 |
| C8 |  | 12 | 89.3 | 266-268 |
| C9 |  | 15 | 54.7 | 271-273 |

*a*:Isolated product yield.

## The antimicrobial activity analysis

Antimicrobial activity evaluation of the obtained compounds **A1**–**A4**, **B1**–**B4** and **C1**–**C9** was carried out using 96-well tissue culture plates. Compounds **A1**–**A4**, **B1**–**B4** and **C1**–**C9** were screened for biological antifungal activities against fungi *C. albicans* ATCC 10231, and resist bacteria l *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922，*S. aureus* ATCC 25923，ATCC 3933 and

*S. epidermidis* ATCC 12228, respectively. Herein, ciprofloxacin (anti-bacteria), fluconazol and itraconazole (anti-fungus) were respectively representatives as positive controls. The results of each tested compound were recorded by the average diameter of inhibition zones for bacteria or fungi cultivated around the discs in mm respectively. Then, the minimum inhibitory concentration (MIC) was measured with the two-fold serial dilution by the method of Clinical and Laboratory Standards Institute (CLSI).[46] All synthesized compounds **A1**–**A4**, **B1**–**B4** and **C1**–**C9** were evaluated under concentrations rang of 0.125–64 μg/mL and the level of the growth inhibition microorganisms had been determined by MIC compared with ciprofloxacin, fluconazol and itraconazole. The data of all the compounds about antifungal and antibacterial activities are displayed in Table **3**. It is seen from Table **3** that some tested compounds exhibited not only antifungal activity but also favorable broad-spectrum antibacterial activity excepted that had no inhibitory effect on *P. aeruginosa*. The tested compounds **A1**–**A4** were almost no inhibition to the tested microorganism when 2-position of thiabendazole or 2-(thiophen-2-yl)-1*H*-benzimidazole(Fig. **1**) was replaced with 3-pyridine ring except from compound A3 (MIC against *C. albicans* was 64 μg/mL). Besides, the tested compounds **B1**–**B4** showed favorable abilities to inhibit bacteria when 2-position of thiabendazole or 2-(thiophen-2-yl)-1*H*-benzimidazole was replaced with 2-oxhydryl-naphtalene ring, the MIC of compound B1 and B4 against *S. aureus* ATCC 25923 and ATCC 3933, and *S. epidermidis* ATCC 12228 respectively were all 16 μg/mL(B1) and 64 μg/mL(B4), the MIC of compound B2 and B3 against *S. aureus* ATCC 3933 and *S. epidermidis* ATCC 12228 were both 32 μg/mL (B2 and B3) and 16 μg/mL (B2 and B3). Only the MIC of compound **B4** against *C. albicans* was 64 μg/mL in **B1**–**B4**. Surprisingly, when the different bromo-substituted thiazole rings were introduced to 2-position of different substituted benzimidazoles, the antifungal and antibacterial activities of compounds **C1**–**C9** were remarkably improved compared with that of compounds **A1**–**A4** and **B1**–**B4**. In the initial exploration, compounds **C3** was found to exhibit the favorable broad-spectrum antimicrobial activities, also, the MICs of compounds **C3** against *C. albicans*, *E. Coli*, *S. aureus* ATCC 25923 and ATCC 3933, and *S. epidermidis* ATCC 12228 were 8 μg/mL, 32 μg/mL, 32 μg/mL, 32 μg/mL and 32 μg/mL, respectively. It was reported halogen substituent on aromatic ring might improve antimicrobial activity.[29][39] Herein, halogen groups were designed to introduce onto benzene ring of benzimidazole and thiazole rings to synthesize new 2,5-disubstituted benzimidazoles(**C4**–**C9**). It was found that the antifungal and antibacterial activities of compounds(**C5** and **C6**) dramatically

enhanced when 3-position of thiazole on 2-position of benzimidazole was substituted by bromo group. Also, the determined MIC of compound **C5** against *S. aureus* ATCC 25923 and ATCC 3933, and *S. epidermidis* ATCC 12228 were respectively 2 μg/mL, 4 μg/mL and 4 μg/mL without antifungal activity compared with that of compound **C3**. This could be because the interaction between chloro substituent on benzene ring of benzimidazole and bromo group on thiazole rings led to no antifungal activity. However, compound **C6** exhibited not only good antifungal activity but also remarkable broad-spectrum antibacterial activity. The MIC against *C. albicans*, *S. aureus* ATCC 25923 and ATCC 3933, and *S. epidermidis* ATCC 12228 respectively were 4 μg/mL, 2 μg/mL, 2 μg/mL and 4 μg/mL. The reason maybe lied in the fact that bromo group on benzene ring of benzimidazole and thiazole rings had good synergistic effect. Unfortunately, the tested compounds **C7**, **C8** and **C9** almost no antimicrobial activities except for the MIC of compound **C8** against *S. aureus* ATCC 25923 when bromo group bearing 4-prisition of thiazole on 2-position of benzimidazole. It suggested that different substituents and their synergies play important roles in the structure-activity relationship of the drug molecular design.

**Table 3** MIC of the synthetic compounds

| Compound No. | MIC (μg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | ***C. albicans*** **ATCC 10231** | ***P. aeruginosa*** **ATCC 27853** | ***E. coli*** **ATCC 25922** | ***S. aureus*** **ATCC 25923** | ***S. aureus*** **ATCC 3933** | ***S. epidermidis*** **ATCC 12228** |
| **A1** | >64 | >64 | >64 | >64 | >64 | >64 |
| **A2** | >64 | >64 | >64 | >64 | >64 | >64 |
| **A3** | **64** | >64 | >64 | >64 | >64 | >64 |
| **A4** | >64 | >64 | >64 | >64 | >64 | >64 |
| **B1** | >64 | >64 | >64 | **16** | **16** | **16** |
| **B2** | >64 | >64 | >64 | >64 | **32** | **16** |
| **B3** | >64 | >64 | >64 | >64 | **32** | **16** |
| **B4** | **64** | >64 | >64 | **64** | **64** | **64** |
| **C1** | >64 | >64 | >64 | >64 | >64 | >64 |
| **C2** | **16** | >64 | **32** | >64 | **64** | **32** |
| **C3** | **8** | >64 | **32** | **32** | **32** | **32** |
| **C4** | **32** | >64 | >64 | **16** | **16** | **16** |
| **C5** | >64 | >64 | >64 | **2** | **4** | **4** |
| **C6** | **4** | >64 | >64 | **2** | **2** | **4** |
| **C7** | >64 | >64 | >64 | >64 | >64 | >64 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **C8** | >64 | >64 | >64 | **4** | >64 | >64 |
| **C9** | >64 | >64 | >64 | >64 | >64 | >64 |
| Ciprofloxacin | - | 0.125 | 0.125 | 0.25 | 0.25 | 0.5 |
| Fluconazole | 0.5 | - | - | - | - | - |
| Itraconazole | 0.125 | - | - | - | - | - |

Note：Values are presented as mean ± SD，n=3

## Conclusion

In a word, a series of 2,5-disubstituted benzimidazole derivatives containing halogen and heterocyclic ring structures using $Na_2S_2O_5$ as the catalyst and oxidant under microwave assisted condition were conveniently synthesized in 50.2–92.5% yields. The effect of various substituted group on the yield of the target products was discussed. The structure of the obtained compounds was confirmed by various spectral such as FT-IR, ESI-MS, $^1$H NMR, $^{13}$C NMR. Further, the test of the antimicrobial activity showed that the synthesized **B1**–**B4** and **C2**–**C6** compounds exhibited not only good antifungal activity but also favorable broad-spectrum antibacterial activity excepted that had no inhibitory effect on *P. aeruginosa*. The MIC of compound C5 and C6 against the tested bacterials were both less than 4 μg/mL, and the lowest one was 2 μg/mL. Besides, The MIC of compound C6 against *C. albicans* was 4 μg/mL. It suggested that different substituent and their synergies play important role in the structure activity relationship of the drug molecular design. In addition, the study for the synthesis of novel benzimidazoles using microwave radiated method and application in antimicrobial and bacterial quorum sensing field is currently in progress in our laboratory.

## Experimental

All the chemical reagents used in the synthesis reactions were available from commercial companies (Tianjin Fuyu Chemical Co. Ltd, China; and Beijing Chemical Plant, China; Aladdin-reagent Co., China) and without further purification. All kinds of analytical-reagent grade of substituted o-phenylenediamine and were from Shanghai Darui Chemical Co. Ltd, China and used without further purification. Thin Layer Chromatography (TLC) was used to tracking the completion of the reaction and silica gel plate (GF 254) was purchased from Qingdao Ocean Chemcial Reagent Company. Silica gel (200-300 mesh) was supplied by Tianjin Fuyu Chemcial Reagent Company. All the melting points were detected by a XT−4 melting point apparatus and were uncorrected. FT-IR spectra were collected, KBr pellets as a reference, using a Shimadzu IRAffinity–1 instrument in the

range of 500−3500 $cm^{-1}$, HRMS were recorded on an Agilent1290–micrOTOF Q II spectrometer. $^{1}H$ NMR and $^{13}C$ NMR spectra was measured using a Bruker AVANCE–600 (Germany) and a Zhongke-Niujin Q. One–AS 400 (China) NMR spectrometer with TMS regard as a reference. The chemical shift $\delta$ concerned with TMS. A XO-50N microwave reactor with a thermocouple thermometer purchased from NanJing Xianou instruments manufacture company was used to synthesize the desired products.

**General synthetic procedures**

A mixture of 4-substituted o-phenylenediamine (1.0 mmol), 3-pyridinecarboxaldehyde (1.1 mmol) or 2-hydroxy-1-naphthaldehyde (1.1 mmol) or substituted thiophene-2-carbaldehyde(1.1 mmol), $Na_2S_2O_5$ ( 1.0 mmol) were dissolved in DMF (15 mL) or EtOH (15 mL) and fully transferred to a 250 mL microwave reaction bottle equipped with a magnetic stir bar, reflux condenser and thermocouple thermoelement, then heated with microwave irradiation at 130 ºC for 10–15 min. The reaction progress was monitored by TLC. When the microwave-assisted reaction was over, the reaction bottle was cooled to room temperature. Further, the residue was poured into beaker filled with 80–100 mL ice water. The crude product was filtrated after it was completely precipitated. The desired pure products **A1-A4**, **B1-B4**, **C1-C9** were obtained by the purified with column chromatography (silica gel, 200-300 mesh) using petroleum ether/ethyl acetate as a developing solvent.

**Representative spectral data**

**5-methyl-2-(pyridin-3-yl)-1*H*-benzimidazole(A1)** Creamy white solid. $^{1}H$ NMR (600 MHz, DMSO-$d_6$) $\delta$, ppm: 12.95 (s, 1H,-NH), 9.35 (d, $J$ = 1.8 Hz, 1H, ArH), 8.71 – 8.63 (m, 1H, ArH), 8.49 (d, $J$ = 8.0 Hz, 1H, ArH), 7.63 – 7.34 (m, 3H, ArH), 7.07 (s, 1H, ArH), 2.45 (s, 3H, $–CH_3$). $^{13}C$ NMR (101 MHz, DMSO) $\delta$, ppm: 150.80, 148.97, 147.88, 134.06, 126.77, 124.45, 21.81. HRMS, m/z: $(M+H)^+$ calcd for $C_{13}H_{11}N_3H$ 210.1026, found 210.1106. IR (KBr) $\upsilon$ ($cm^{-1}$): 3120, 3039, 3001, 2947, 2866, 1624, 1581, 1527, 1492, 1450, 1315, 1226, 1273, 952, 810, 705.

**2-(pyridin-3-yl)-1*H*-benzimidazole(A2)** Yellow solid. $^{1}H$ NMR (600 MHz, DMSO-$d_6$) $\delta$, ppm:13.12 (s, 1H, -NH), 9.38 (d, $J$ = 2.3 Hz, 1H, ArH), 8.69 (dd, $J$ = 4.8, 1.5 Hz, 1H, ArH), 8.52 (dd, $J$ = 8.0, 1.9 Hz, 1H, ArH), 7.71 – 7.57 (m, 3H, ArH), 7.28 – 7.23 (m, 2H, ArH).$^{13}C$ NMR (101 MHz, DMSO) $\delta$, ppm: 151.00, 149.34, 148.01, 134.23, 126.65, 124.50. HRMS, m/z: $(M+H)^+$ calcd for

$C_{12}H_9N_3H$ 196.0869, found 196.0958. IR (KBr) $\upsilon$ ($cm^{-1}$): 3441, 3043, 2993, 2912, 2877, 1573, 1539, 1489, 1446, 1273, 1226, 1188, 1022, 867, 810, 740, 698.

**5-chloro-2-(pyridin-3-yl)-1*H*-benzimidazole(A3)** Creamy white solid. $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$, ppm:13.32 (s, 1H, –NH), 9.36 (dd, $J$ = 2.3, 0.9 Hz, 1H, ArH), 8.71 (dd, $J$ = 4.8, 1.6 Hz, 1H, ArH), 8.50 (dt, $J$ = 7.9, 1.9 Hz, 1H, ArH), 7.72 (d, $J$ = 8.7 Hz, 1H, ArH), 7.69 – 7.63 (m, 1H, ArH), 7.61 (ddd, $J$ = 8.0, 4.8, 0.9 Hz, 1H, ArH), 7.27 (dd, $J$ = 8.5, 2.0 Hz, 1H, ArH). $^{13}$C NMR (101 MHz, DMSO) $\delta$, ppm: 151.31, 150.77, 148.10, 134.40, 126.22, 124.53. HRMS, m/z: $(M+H)^+$ calcd for $C_{12}H_8ClN_3H$ 230.0480, found 230.0483. IR (KBr) $\upsilon$ ($cm^{-1}$): 3491, 3124, 3032, 2954, 1577, 1523, 1446, 1396, 1276, 1222, 1188, 1056, 844, 810, 702.

**5-bromo-2-(pyridin-3-yl)-1*H*-benzimidazole(A4)** Brown schistose solid. $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$, ppm:13.31 (d, $J$ = 24.0 Hz, 1H, -NH), 9.35 (dd, $J$ = 2.3, 0.9 Hz, 1H, ArH), 8.71 (dd, $J$ = 4.8, 1.6 Hz, 1H, ArH), 8.50 (ddd, $J$ = 8.0, 2.3, 1.7 Hz, 1H, ArH), 7.69 (d, $J$ = 8.17 Hz, 1H, ArH), 7.60 (dd, $J$ = 4.8, 0.9 Hz, 1H, ArH), 7.42 – 7.35 (m, 1H, ArH).$^{13}$C NMR (101 MHz, DMSO) $\delta$, ppm: 151.33, 150.75, 148.13, 134.43, 126.19, 124.53. HRMS, m/z: $(M+H)^+$ calcd for $C_{12}H_8BrN_3H$ 273.9974, found 273.9990. IR (KBr) $\upsilon$ ($cm^{-1}$): 3444, 3124, 2981, 2951, 2900, 1573, 1539, 1485, 1442, 1276, 1222, 1184, 1041, 910, 806, 752, 698.

**1-(*5*-methyl-1*H*-benzimidazol-2-yl)naphthalen-2-ol(B1)** Faint yellow sliod. $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$, ppm: 12.29 (s, 1H, –NH), 8.26 (dd, J = 8.5, 1.0 Hz, 1H, ArH), 7.99 – 7.93 (m, 1H, ArH), 7.93 – 7.88 (m, 1H, ArH), 7.59 – 7.51 (m, 2H, ArH), 7.46 (s, 1H, ArH), 7.39 (ddd, J = 8.0, 6.8, 1.1 Hz, 1H, ArH), 7.33 (d, J = 8.9 Hz, 1H, ArH), 7.09 (dd, J = 8.1, 1.7 Hz, 1H, ArH), 2.47 (s, 3H, –$CH_3$). $^{13}$C NMR (101 MHz, DMSO) $\delta$, ppm: 155.14, 142.13, 137.52, 135.85, 133.31, 129.70, 128.34, 127.29, 123.75, 121.65, 120.85, 119.32, 118.64, 116.80, 109.72, 21.42. HRMS, m/z: $(M+H)^+$ calcd for $C_{18}H_{14}N_2OH$ 275.1179, found 275.1157. IR (KBr) $\upsilon$ ($cm^{-1}$):3325, 3132, 3045, 2993, 2984, 1615, 1530, 1466, 1345, 1268, 889, 856, 744.

**1-(1*H*-benzimidazol-2-yl)naphthalen-2-ol(B2)** Faint yellow sliod. $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$, ppm: 12.24 (s, 2H, –NH, –OH), 8.24 (d, J = 8.6, 1H, ArH), 7.96 (d, J = 8.9 Hz, 1H, ArH), 7.91 (d, J = 7.9 Hz, 1H, ArH), 7.67 (dd, J = 5.1, 3.3 Hz, 2H, ArH), 7.53 (ddd, J = 8.4, 6.8, 1.4 Hz, 1H, ArH), 7.38 (m, 2H, ArH), 7.28 (dd, J = 8.5, 2.1 Hz, 1H, ArH). $^{13}$C NMR (101 MHz, DMSO) $\delta$, ppm: 156.12, 149.84, 132.52, 132.28, 128.88, 128.41, 127.86, 124.58, 123.72, 122.54, 119.04, 108.93. HRMS, m/z: $(M+H)^+$ calcd for $C_{17}H_{12}ClN_2OH$ 261.1022, found 261.1162. IR (KBr) $\upsilon$ ($cm^{-1}$):

3322, 3122, 3040, 1615, 1521, 1456, 1334, 1268, 893, 856, 734.

**1-(*5*chloro-1*H*-benzimidazol-2-yl)naphthalen-2-ol(B3)** Yellow solid. $^{1}$H NMR (600 MHz, DMSO-d6) $\delta$, ppm: 12.04 (s, 2H, –NH, –OH), 8.24 (dd, J = 8.6, 1.1 Hz, 1H, ArH), 7.97 (d, J = 8.9 Hz, 1H, ArH), 7.90 (dd, J = 8.2, 1.3 Hz, 1H, ArH), 7.71 (dd, J = 36.1, 9.1 Hz, 2H, ArH), 7.51 (ddd, J = 8.4, 6.8, 1.4 Hz, 1H, ArH), 7.40 – 7.34 (m, 2H, ArH), 7.28 (dd, J = 8.5, 2.1 Hz, 1H, ArH). $^{13}$C NMR (101 MHz, DMSO) $\delta$, ppm: 153.47, 150.11, 147.19, 126.45, 122.89, 122.36, 118.62, 114.27, 112.62. HRMS, m/z: $(M+H)^+$ calcd for $C_{17}H_{12}ClN_2OH$ 295.0633, found 295.0762. IR (KBr) $\upsilon$ ($cm^{-1}$): 3342, 3162, 3047, 1615, 1528, 1456, 1354, 1278, 890, 876, 831, 740.

**1-(5-bromo-1*H*-benzimidazol-2-yl)naphthalen-2-ol(B4)** Yellow solid. $^{1}$H NMR (600 MHz, DMSO-$d_6$) $\delta$, ppm: 11.99 (s, 1H, –NH), 8.27 – 8.19 (m, 1H, ArH), 7.97 (d, J = 8.9 Hz, 1H, ArH), 7.90 (dd, J = 8.2, 1.4 Hz, 1H, ArH), 7.73 (s, 1H, ArH), 7.67 (d, J = 8.5 Hz, 1H, ArH), 7.51 (ddd, J = 8.4, 6.8, 1.4 Hz, 1H, ArH), 7.40 – 7.34 (m, 2H, ArH), 7.28 (dd, J = 8.5, 2.1 Hz, 1H, ArH). $^{13}$C NMR (101 MHz, DMSO) $\delta$, ppm: 155.78, 150.99, 132.80, 132.45, 128.82, 128.41, 127.87, 126.73, 124.72, 123.78, 122.61, 118.90, 109.16. HRMS, m/z: $(M+H)^+$ calcd for $C_{17}H_{12}BrN_2OH$ 339.0128, found 339.0154. IR (KBr) $\upsilon$ ($cm^{-1}$): 3339, 3146, 3030, 1635, 1551, 1436, 1324, 1265, 893, 866, 737, 660.

5-**methyl-2-(thiophen-2-yl)-1*H*-benzimidazole(C1)** Faint yellow solid. $^{1}$H NMR (600 MHz, DMSO-$d_6$) $\delta$, ppm: 12.79 (d, $J$ = 13.3 Hz, 1H,–NH), 7.81 (d, $J$ = 3.6 Hz, 1H, ArH), 7.70 (d, $J$ = 5.0 Hz, 1H, ArH), 7.53 – 7.26 (m, 2H, ArH), 7.22 (dd, $J$ = 5.0, 3.7 Hz, 1H, ArH), 7.06 – 6.97 (m, 1H, ArH), 2.42 (s, 3H, –$CH_3$). $^{13}$C NMR (101 MHz, DMSO) $\delta$, ppm: 147.14, 134.41, 128.92, 128.69, 126.85, 123.73, 111.33, 21.81. HRMS, m/z: $(M+H)^+$ calcd for $C_{12}H_{10}N_2SH$ 215.0637, found 215.0731. IR (KBr) $\upsilon$ ($cm^{-1}$): 3336, 3035, 3016, 2924, 1566, 1492, 1462, 1419, 1280, 1230, 1130, 1072, 941, 852, 798, 713, 597.

**5-chloro-2-(thiophen-2-yl)-1*H*-benzimidazole(C2)** Yellow schistose solid. $^{1}$H NMR (600 MHz, DMSO-$d_6$) $\delta$, ppm: 13.13 (s, 1H, –NH), 7.85 (dd, $J$ = 3.7, 1.1 Hz, 1H, ArH), 7.75 (dd, $J$ = 5.0, 1.1 Hz, 1H, ArH), 7.55 (s, 2H, ArH), 7.23 (dd, $J$ = 5.0, 3.7 Hz, 1H, ArH), 7.20 (dd, $J$ = 8.6, 2.0 Hz, 1H, ArH). $^{13}$C NMR (101 MHz, DMSO) $\delta$, ppm: 145.09, 135.97, 133.61, 128.87, 123.12, 112.88. HRMS, m/z: $(M+H)^+$ calcd for $C_{11}H_7ClN_2SH$ 235.0091, found 235.0109. IR (KBr) $\upsilon$ ($cm^{-1}$): 3325, 3062, 3001, 1566, 1492, 1465, 1438, 1338, 1296, 1230, 1060, 852, 798, 597.

**5-bromo-2-(thiophen-2-yl)-1*H*-benzimidazole(C3)** Creamy white schistose solid. $^{1}$H NMR (600 MHz, DMSO-$d_6$) $\delta$, ppm: 13.14 (s, 1H, –NH), 7.86 (dd, $J$ = 3.7, 1.2 Hz, 1H, ArH), 7.77 (dd, $J$ =

5.0, 1.2 Hz, 1H, ArH), 7.52 (s, 1H, ArH), 7.33 (dd, *J* = 8.5, 1.9 Hz, 1H, ArH), 7.25 (dd, *J* = 5.0, 3.7 Hz, 1H, ArH). $^{13}$C NMR (101 MHz, DMSO) *δ*, ppm: 145.62, 136.47, 134.32, 133.53, 128.87, 114.39. HRMS, m/z: $(M+H)^+$ calcd for $C_{11}H_7BrN_2SH$ 278.9586, found 278.9937. IR (KBr) *υ* ($cm^{-1}$): 3305, 3062, 2993, 2912, 1566, 1492, 1462, 1415, 1276, 1234, 1095, 941, 914, 852, 802, 709, 590.

**2-(3-bromothiophen-2-yl)-5-methyl-1*H*-benzimidazole(C4)** Creamy white solid. $^1$H NMR (500 MHz, DMSO-$d_6$) *δ*, ppm: 12.87 (s, 1H, –NH), 7.62 (d, *J* = 3.9 Hz, 1H, ArH), 7.45 (d, *J* = 8.9 Hz, 1H, ArH), 7.35 (d, *J* = 3.9 Hz, 2H, ArH), 7.03 (dd, *J* = 8.4, 1.6 Hz, 1H, ArH), 2.42 (s, 3H, $–CH_3$). $^{13}$C NMR (101 MHz, DMSO) *δ*, ppm: 146.07, 136.16, 132.13, 127.28, 124.67, 114.35, 21.81. HRMS, m/z: $(M+H)^+$ calcd for $C_{12}H_9BrN_2SH$ 292.9743, found 292.9750. IR (KBr) *υ* ($cm^{-1}$): 3329, 3039, 3026, 2934, 1556, 1482, 1452, 1429, 1278, 1235, 1125, 1062, 931, 842, 788, 723, 607.

**2-(3-bromothiophen-2-yl)-5-chloro-1*H*-benzimidazole(C5)** Yellow solid. $^1$H NMR (600 MHz, DMSO-$d_6$) *δ*, ppm: 13.22 (s, 1H, –NH), 7.67 (d, *J* = 4.0 Hz, 1H, ArH), 7.63 (s, 1H, ArH), 7.58 (d, *J* = 8.5 Hz, 1H, ArH), 7.38 (d, *J* = 4.0 Hz, 1H, ArH), 7.23 (dd, *J* = 8.6, 2.1 Hz, 1H, ArH). $^{13}$C NMR (101 MHz, DMSO) *δ*, ppm: 147.70, 135.32, 132.29, 128.22, 127.14, 123.21, 115.29. HRMS, m/z: $(M+H)^+$ calcd for $C_{11}H_6BrClN_2SH$ 312.9196, found 312.9206. IR (KBr) *υ* ($cm^{-1}$): 3324, 3082, 3021, 1586, 1502, 1455, 1448, 1348, 1292, 1220, 1050, 842, 795, 590.

**5-bromo-2-(3-bromothiophen-2-yl)-1*H*-benzimidazole(C6)** Brown solid. $^1$H NMR (600 MHz, DMSO-$d_6$) *δ*, ppm: 13.22 (s, 1H, –NH), 7.79 – 7.74 (m, 1H, ArH), 7.67 (d, *J* = 3.9 Hz, 1H, ArH), 7.53 (d, *J* = 8.4 Hz, 1H, ArH), 7.38 (d, *J* = 4.0 Hz, 1H, ArH), 7.35 (dd, *J* = 8.5, 1.9 Hz, 1H, ArH). $^{13}$C NMR (101 MHz, DMSO) *δ*, ppm: 147.53, 135.28, 132.31, 128.28, 125.78, 115.33. HRMS, m/z: $(M+H)^+$ calcd for $C_{11}H_6Br_2N_2SH$ 356.8691, found 356.8699. IR (KBr) *υ* ($cm^{-1}$): 3320, 3052, 2995, 2922, 1566, 1482, 1453, 1410, 1266, 1235, 1085, 945, 916, 855, 812, 719, 595.

**2-(4-bromothiophen-2-yl)-5-methyl-1*H*-benzimidazole(C7).** Creamy white solid. $^1$H NMR (600 MHz, DMSO-$d_6$) *δ*, ppm: 12.88 (s, 1H, –NH), 7.84 (d, *J* = 1.5 Hz, 1H, ArH), 7.79 (d, *J* = 1.5 Hz, 1H, ArH), 7.47 (d, *J* = 7.6 Hz, 1H, ArH), 7.37 (s, 1H, ArH), 7.06 – 7.03 (m, 1H, ArH), 2.43 (s, 3H, $–CH_3$). $^{13}$C NMR (101 MHz, DMSO) *δ*, ppm: 145.79, 135.91, 128.57, 126.42, 124.48, 109.81, 21.80. HRMS, m/z: $(M+H)^+$ calcd for $C_{12}H_9BrN_2SH$ 292.9743, found 292.9754. IR (KBr) *υ* ($cm^{-1}$): 3340, 3039, 3010, 2934, 1556, 1497, 1472, 1423, 1275, 1225, 1130, 1062, 972, 842, 786, 723, 591.

**2-(4-bromothiophen-2-yl)-5-chloro-1*H*-benzimidazole(C8)** Faint yellow solid. $^1$H NMR (600 MHz, DMSO-$d_6$) *δ*, ppm: 13.21 (s, 1H, –NH), 7.89 (d, *J* = 1.5 Hz, 1H, ArH), 7.84 (d, *J* = 1.5 Hz, 1H,

ArH), 7.67 – 7.64 (m, 1H, ArH), 7.60 (d, $J$ = 8.6 Hz, 1H, ArH), 7.25 (dd, $J$ = 8.6, 2.1 Hz, 1H, ArH). $^{13}$C NMR (101 MHz, DMSO) $\delta$, ppm: 147.43, 135.11, 129.45, 127.25, 123.18, 109.98. HRMS, m/z: $(M+H)^+$ calcd for $C_{11}H_6BrClN_2SH$ 312.9196, found 312.9210. IR (KBr) $\upsilon$ ($cm^{-1}$): 3325，3052，3005，1567, 1482, 1455, 1408, 1331, 1285, 1220, 1051, 852, 799, 587.

**5-bromo-2-(4-bromothiophen-2-yl)-1*H*-benzimidazole(C9)** Brown solid. $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$, ppm: 13.22 (s, 1H, –NH), 7.90 (d, $J$ = 1.4 Hz, 1H, ArH), 7.85 (d, $J$ = 1.5 Hz, 1H, ArH), 7.79 (s, 1H, ArH), 7.55 (d, $J$ = 8.5 Hz, 1H, ArH), 7.36 (dd, $J$ = 8.6, 1.9 Hz, 1H, ArH). $^{13}$C NMR (101 MHz, DMSO) $\delta$, ppm: 147.25, 135.05, 129.49, 127.29, 125.94, 109.99. HRMS, m/z: $(M+H)^+$ calcd for $C_{11}H_6Br_2N_2SH$ 356.8691, found 356.8699. IR (KBr) $\upsilon$ ($cm^{-1}$): 3315, 3062, 2990, 2914, 1564, 1498, 1465, 1418, 1274, 1224, 1090, 944, 916, 855, 805, 709, 595.

**Biological activity analysis**

The antibacterial activity was evaluated by the antimicrobial susceptibility test method of Clinical and Laboratory Standards Institute (CLSI) in vitro[46]. Bacteria strains were cultured to 6-8 h at 37 °C in Mueller-Hinton broth (MHB) for bacteria strains. Meanwhile, yeast strains were incubated to 24 h at 35 °C in yeast extract peptone dextrose medium (YEPD). The range of inoculum density was limited to 2–4×$10^8$ CFU/mL (about two times of the final inoculum concentration) for bacteria, while yeast was range from 1.0×$10^3$ to 5.0×$10^3$ CFU/mL(two times about the final inoculum concentration). Herein, fluconazole and itraconazole were chosen as positive controls against fungi and ciprofloxacin was chosen as positive controls against bacteria.

Compounds were dissolved in dimethylsulfoxide(DMSO) solution at 5.12 mg/mL. Tests were accomplished in sterile 96-well plates, which were added to 100 μL prepared inoculum into each well. Meanwhile, another 100 μL inoculum containing 2 μL of test compound was added into the first well and mixed well. Then obtained 100 μL mixed inoculum from the first well was transfered into the second well. Therefore, this two-fold dilution method was sustained from the second to the ninth well, and so on. The 100μL inoculum of the ninth column was discarded. The tenth well was served as blank control (without inoculation), the eleventh column was as negative growth control (with standard antimicrobial medicines) and the twelfth column was as positive growth control (without medicines or test compounds). The final concentrations of the compounds were ranged from 64 μg/mL to 0.125 μg/mL and DMSO concentration was not exceeding 1% V/V in each microplate.

The bacteria strains of plates were covered and cultivated for 18 h at 37 °C, while yeast for 24 h at 30 °C. The MIC was defined as the lowest concentration which inhibits the visible growth of a microbe.

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## The Author Contribution Statement

**Yanpeng Shi** performed the synthesis of experiments, analyzed the data and wrote the paper.

**Kai Jiang** performed the antimicrobial activity evaluation of the obtained compounds.

**Ran Zheng, Jiaxu Fu and Liuqing Yan** performed the synthesis of some compounds.

**Qiang Gu** designed some experiments.

**Yumin Zhang** conceived and designed the experiments, explained experimental phenomena and laws, and revised the manuscript.

**Feng Lin** conceived and designed the antimicrobial experiments

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