Discovery of New Imidazole Derivatives Containing the 2,4-Dienone Motif with Broad-Spectrum Antifungal and Antibacterial Activity

Chunli Liu 1,†, Ce Shi 2,3,†, Fei Mao 1,*, Yong Xu 4, Jinyan Liu 2, Bing Wei 2,3, Jin Zhu 1, Mingjie Xiang 2,3,* and Jian Li 1,*

1 Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, 130 Mei Long Road, Shanghai 200237, China
2 Radioimmunology and Clinical Laboratory, Luwan Branch, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200020, China
3 Department of Laboratory Medicine, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200025, China
4 Humanwell Healthcare (Group) Co, Ltd., 666 Gaoxin Road, East Lake High-Tech Development Zone, Wuhan 430075, China

† These authors contributed equally to this work.

* Authors to whom correspondence should be addressed; E-Mails: maofei6517@163.com (F.M.); mjxiang123456@126.com (M.X.); jianli@ecust.edu.cn (J.L.);
Tel./Fax: +86-21-6425-2584 (F.M. & J.L.); Tel.: +86-21-6437-0045 (M.X.);
Fax: +86-21-6386-7643 (M.X.).

External Editor: Derek J. McPhee

Received: 14 August 2014; in revised form: 14 September 2014 / Accepted: 18 September 2014 / Published: 29 September 2014

Abstract: A compound containing an imidazole moiety and a 2,4-dienone motif with significant activity toward several fungi was discovered in a screen for new antifungal compounds. Then, a total of 26 derivatives of this compound were designed, synthesized and evaluated through in vitro and in vivo antifungal activity assays. Several compounds exhibited improved antifungal activities compared to the lead compound. Of the derivatives, compounds 31 and 42 exhibited strong, broad-spectrum inhibitory effects toward Candida spp. In particular, the two derivatives exhibited potent antifungal activities toward the fluconazole-resistant isolate C. albicans 64110, with both having MIC values of
8 µg/mL. In addition, they had significant inhibitory effects toward two Gram-positive bacteria, *Staphylococcus aureus* UA1758 (compound 31: MIC = 8 µg/mL; compound 42: MIC = 4 µg/mL) and *Staphylococcus epidermidis* UF843 (compound 31: MIC = 8 µg/mL; compound 42: MIC = 8 µg/mL). The results of an animal experiment indicated that both compounds could improve the survival rate of model mice infected with ATCC 90028 (fluconazole-susceptible isolate). More importantly, the two compounds exhibited notable *in vivo* effects toward the fluconazole-resistant *C. albicans* isolate, which is promising with regard to the clinical problem posed by fluconazole-resistant *Candida* species.

**Keywords:** imidazole; 2,4-dienone; broad-spectrum; antifungal; antibacterial

1. Introduction

Infections caused by bacteria and fungi lead to diseases and an enormous social burden as millions of people are infected by bacteria and fungi every year worldwide. Therefore, a large number of antimicrobial drugs have been listed, which play an important role in treating infections [1]. As the need for antifungal intervention has increased, so too has the prevalence of resistance [2]. With the irrational use of antibiotics, the resistance of microorganisms has become a very serious clinical problem. Growing antifungal resistance poses the threat that there will be no available drugs for the treatment of common infections in the future [3], so there is an urgent need for the discovery of new compounds with antibacterial and antifungal activities [4], especially those with mechanisms of action that are distinct from the well-known classes of antifungal agents [5–7].

The development of derivatives based on heterocyclic scaffolds is a fast emerging subject in medicinal chemistry. Azole compounds in particular play a remarkably important role in the field of medicinal chemistry. A great deal of azole-based antibacterial and antifungal agents have been extensively studied as drug candidates, and some of them have been used in the clinic, for instance itraconazole, fluconazole, posaconazole and voriconazole, which suggests the great development value of azole compounds [8]. However, the reduced susceptibility of *Cryptococcus neoformans* to fluconazole has been reported in a number of sub-Saharan countries including Kenya, Uganda, Rwanda and South Africa [9]. As a result, there is an unmet need for the development of new azole antifungals.

Imidazole compounds containing two nitrogen atoms in a five-membered aromatic azole ring have received special attention in recent years. As reported, imidazole rings are widely employed as spin-trapping species in the interesting application of designing drugs with neuroprotective activity [10,11]. These imidazole-based derivatives also have several favorable properties such as excellent bioavailability, good tissue penetrability and permeability and a relatively low incidence of adverse and toxic effects, which suggests that they have considerable development potential in chemistry, materials science and medicinal chemistry [12]. Their extensive applications, especially as antifungal agents, are frequently investigated, and this has become one of the most active areas in antifungal drug development. Many imidazole-based derivatives have been marketed as antifungal drugs such as ketoconazole (1), miconazole (2), clotrimazole (3), tioconazole (4), econazole (5), tinidazole (6), enilconazole/imazalil (7), parconazole (8), eberconazole (9), lanoconazole (10), fenticonazole (11),
bifonazole (12), sulconazole (13), lombazole (14), and sertaconazole (15) (Figure 1) [13–19], which indicates their large development value and broad potential as antifungal agents.

**Figure 1.** Marketed imidazole drugs.

The imidazole ring has been demonstrated to be a versatile core of many biologically active molecules, especially those with antifungal properties. Thus, we screened the imidazole-based compounds in our inventory. As a result, compound 24 in Figure 2 was discovered in our screening to exhibit antifungal activity.

**Figure 2.** The structural scaffold of the lead compound synthesized in this study 24 and structurally similar compounds 16–17 reported in the literature.
The MIC values of this compound toward Candida albicans ATCC 90028, Candida glabrata 923, Candida glabrata 168 and Candida parapsilosis 27 were 2, 4, 4 and 16 µg/mL, respectively, but this compound was found to be inactive against Candida albicans 205, Candida albicans 64110, Candida krusei ATCC 6528, and Candida tropicalis 657. Considering the biological potency and synthetic accessibility of compound 24, it has the potential to serve as a reasonable lead compound for further development.

From the viewpoint of a medicinal chemist, the chemical structure of this compound contains two distinctive moieties: a 2-(1H-imidazol-1-yl)-1-phenylethanone moiety and an (E)-buta-1,3-dien-1-ylibenzene moiety (24 in Figure 2). A literature survey indicates that compounds containing such moieties exhibit antifungal activities. For example, a number of 2-(1H-imidazol-1-yl)-1-phenylethanone derivatives (for example 16 in Figure 2) exhibit powerful activities against C. albicans and P. chrysogenum, but moderate activity against A. niger at a concentration of 10 µg/mL [20]. In addition, (E)-2-((E)-1-methoxy-3-phenylallylidene)-4-methylcyclopent-4-ene-1,3-dione (17 in Figure 2) exhibited varying degrees of antifungal activity against fungi C. albicans ATCC 90028 (2.08 µg/mL), C. neoformans ATCC 90113 (8.33 µg/mL) and A. fumigatus ATCC 90906 (>20 µg/mL) [21]. Given these facts, it is reasonable to expect that a compound combining these two moieties may have antifungal activities. Therefore, the active compound identified in our screening, compound 24, is worthy of further development. Based on this information, in this study, we designed, synthesized and evaluated a series of derivatives 21–46 of compound 24 and evaluated their activities as antifungal and antibacterial agents.

2. Results and Discussion

2.1. Chemistry

The synthetic methods used to prepare compounds 21–46 are illustrated in Scheme 1. The non-commercial intermediates (compounds 19a, 19k, 19m, 19n, 19o, 19q, 19r, 19s) were synthesized by irradiating the appropriate acetophenone in the presence of Br2 at a power of 250 W for 5 h (Scheme 1). Then, compound 20, the key intermediate, was obtained through the reaction of intermediate 19 with imidazole. Finally, various aldehydes were reacted with the corresponding intermediate 20 to produce the target compounds 21–44 and 46 via a Knoevenagel reaction. There was an interconversion between the E and Z isomers in the reaction solvent, which complicated the purification of the products. The ultimate solution was to purify the compounds by recrystallization from a mixed solvent of ethyl acetate and petroleum ether (v/v, 1/70) after silica gel column chromatography. The two-dimensional hydrogen spectrum suggested that the obtained compounds had only the Z-configuration.

To investigate the influence of the carbonyl moiety, compound 45 was also synthesized by reducing compound 31 with sodium borohydride, as shown in Scheme 1. All compounds were fully analyzed and characterized by 1H nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HRMS) before biological evaluation.
Scheme 1. The synthetic routes used to prepare compounds 21–46.

Reagents and conditions: (a) Br₂, glacial acetic acid, 48% hydrogen bromide, 250 W, 5 h; (b) triethylamine, tetrahydrofuran, r.t., overnight; (c) piperidine, glacial acetic acid, toluene, 75 °C, 4 h; (d) NaBH₄, anhydrous methanol, r.t.

2.2. Antifungal Susceptibility Assay

All of the synthesized compounds were evaluated using in vitro antifungal activity assays against Candida albicans ATCC 90028, Candida albicans 205, Candida albicans 64110, Candida krusei ATCC 6528, Candida tropicalis 657, Candida glabrata 923, Candida glabrata 168 and Candida parapsilosis 27 (Table 1). The minimum inhibitory concentrations (MICs) of 26 compounds and fluconazole (Pfizer Pharmaceuticals, Shanghai, China) for the tested isolates were determined based on the standard guidelines described in the Clinical and Laboratory Standards Institute document M27-A3 using the microdilution reference method.
The MICs of all antifungal agents against the tested strains of *Candida* spp. determined by broth microdilution are listed in Table 1. The MICs of most compounds were higher than those of fluconazole, while compounds 31 and 42 exhibited more potent inhibitory effects on the tested *Candida* spp. In particular, the two derivatives showed potent antifungal activity toward the fluconazole-resistant isolate *C. albicans* 64110, with a MIC value of 8 µg/mL, which was a relatively excellent result.

### 2.3. Conclusion of the Structure-Activity Relationship

SAR of this class of compounds has been well studied through variation of the styryl, imidazole and benzoyl moieties, as shown in Table 1. First, the substituents on the A-ring were varied to improve the antifungal activity of the compounds. Thus, the first batch of compounds (21–23, 25, 26) was synthesized by replacing the 4-Br (24 in Figure 2) with 4-NO₂, 2-NO₂, 4-F, 4-OCH₃, and 2,4-diCl. Unfortunately, all of the resulting derivatives exhibited weaker antifungal activities than the lead compound 24. Both 2-substitution (22, 26) and electron-donating groups (25) decreased the antifungal activity.

With the second batch of compounds (27 and 28), the effect of the imidazole moiety was investigated. The antifungal activities were largely abolished when the imidazole was replaced with 4-methyl-1H-imidazole or 4,5-dichloro-1H-imidazole.

For the third batch of compounds, sixteen compounds (29–44) were obtained and eight (30, 31, 36, 39, 42, 43, 45, 46) of them exhibited noticeable antifungal activities. Among them, compounds 31 and 42 exhibited broad-spectrum antifungal activities with MIC values of 0.5–8 µg/mL and 2–32 µg/mL, respectively. Replacement of benzoyl with p-fluorobenzoyl or pyridin-2-yl-formyl was apparently beneficial with respect to their antifungal activities. In particular, 4-fluoro derivative 31 exhibited potent antifungal activity toward the fluconazole-resistant isolate *C. albicans* 64110, with a MIC value of 8 µg/mL. Increasing the electronegativity of the substituent (34) or the volume occupied by it (29, 30, 41) and introducing electron-donating groups (32, 33) or second substituents (35–40) resulted in relatively poor inhibitory activities. The results above indicated that substitution of fluoro in the para position of the phenyl group was essential for maintaining the antifungal activity.

Furthermore, the antifungal activities of compounds 45 and 46 were also explored. Both of them exhibited a significant decrease in activity, which suggested that the conjugated double bonds and carbonyl were important for the activity.

### 2.4. Antibacterial Susceptibility Assay

The antibacterial activities of the two compounds (compounds 31 and 42) with excellent antifungal activities were also evaluated by antibacterial activity assays. The minimum inhibitory concentrations (MICs) of the two compounds were determined based on standard guidelines described in the Clinical and Laboratory Standards Institute document M27-A3 using the broth microdilution reference method. The results are shown in Table 2. Among the tested strains, the two compounds were effective toward two Gram-positive bacteria, *Staphylococcus aureus* UA1758 (compound 31: MIC = 8 µg/mL; compound 42: MIC = 4 µg/mL) and *Staphylococcus epidermidis* UF843 (compound 31: MIC = 8 µg/mL; compound 42: MIC = 8 µg/mL).
Table 1. Chemical structures of compounds 21–46 and their antifungal activities.

| Compd. | R¹ | R² | Ar        | C. albicans ATCC 90028 | C. albicans 205 | C. albicans 64110 | C. krusei ATCC 6528 | C. tropicalis 657 | C. Glabrata 923 | C. Glabrata 186 | C. parapsilosis 27 |
|--------|----|----|-----------|------------------------|----------------|-------------------|----------------------|-----------------|----------------|----------------|------------------|
| 21     | H  | 4-NO₂ | phenyl   | >128                   | >128           | >128              | >128                 | >128            | 8              | 16             | >128             |
| 22     | H  | 2-NO₂ | phenyl   | >128                   | >128           | >128              | >128                 | >128            | >128           | >128           | >128             |
| 23     | H  | 4-F   | phenyl   | 32                     | >128           | >128              | >128                 | >128            | >128           | >128           | >128             |
| 24     | H  | 4-Br  | phenyl   | 2                      | >128           | >128              | >128                 | >128            | 4              | 4              | 16              |
| 25     | H  | 4-MeO | phenyl   | >128                   | >128           | >128              | >128                 | >128            | >128           | >128           | >128             |
| 26     | H  | 2,4-diCl | phenyl | >128                   | >128           | >128              | >128                 | >128            | >128           | >128           | >128             |
| 27     | 4,5-diCl | 4-Br | phenyl   | >128                   | >128           | >128              | >128                 | >128            | >128           | >128           | >128             |
| 28     | 4-CH₃ | 4-Br | phenyl   | >128                   | >128           | >128              | >128                 | >128            | >128           | >128           | >128             |
| 29     | H  | 4-Br  | 4-Br-phenyl | 8                     | >128           | >128              | >128                 | >128            | 2              | 4              | 2               |
| 30     | H  | 4-Br  | 4-Cl-phenyl | 2                     | >128           | >128              | >128                 | >128            | 4              | 8              | 8               |
| 31     | H  | 4-Br  | 4-F-phenyl | 0.5                | 4              | 8                 | 8                    | 2               | 2              | 2              | 4               |
| 32     | H  | 4-Br  | 4-Me-phenyl | 2                     | >128           | >128              | >128                 | >128            | >128           | >128           | >128             |
| 33     | H  | 4-Br  | 4-MeO-phenyl | >128              | >128           | >128              | >128                 | >128            | >128           | >128           | >128             |
| 34     | H  | 4-Br  | 4-CN-phenyl | 2                     | >128           | >128              | >128                 | 4               | 1              | 1              | 4               |
| 35     | H  | 4-Br  | 2,4-diCl-phenyl | 2                    | >128           | >128              | >128                 | 2               | >128           | >128           | >128             |
| 36     | H  | 4-Br  | 3,4-diF-phenyl | 2                    | >128           | >128              | 16                    | 8               | 8              | 16             |                 |
| 37     | H  | 4-Br  | 3-Cl-4-F-phenyl | >128               | >128           | >128              | >128                 | 32              | 32             | 32             | >128            |
| 38     | H  | 4-Br  | 3-Br-4-F-phenyl | >128               | >128           | >128              | >128                 | >128            | >128           | >128           | >128             |
| 39     | H  | 4-Br  | 2,4-diF-phenyl | 0.5                | >128           | >128              | >128                 | 16              | 32             | 32             | 8               |
| 40     | H  | 4-Br  | 2-NO₂-4-F-phenyl | 8                   | >128           | >128              | >128                 | >128            | >128           | >128           | >128             |
| 41     | H  | 4-Br  | 1,1'-biphenyl | >128               | >128           | >128              | >128                 | >128            | >128           | >128           | >128             |
| 42     | H  | 4-Br  | pyridin-2-yl   | 4                    | 8              | 8                 | 32                    | 2               | 4              | 8              | 8               |
| 43     | H  | 4-Br  | 5-F-pyridin-2-yl | 4                    | 8              | 32                | 64                    | 2               | 64             | 64             | 1               |
| 44     | H  | 4-Br  | furan-2-yl    | >128                 | >128           | >128              | >128                 | >128            | >128           | >128           | >128             |
### Table 1. Cont.

| Compd. | R¹ | R² | Ar | C. albicans ATCC 90028 | C. albicans 205 | C. albicans 64110 | C. krusei ATCC 6528 | C. tropicalis 657 | C. Glabrata 923 | C. Glabrata 186 | C. parapsilosis 27 |
|--------|----|----|----|------------------------|---------------|-------------------|-------------------|----------------|----------------|----------------|-----------------|
| 45     | ![Structure](image) | | | 8 | 128 | 128 | 128 | 128 | 128 | 128 | 32 |
| 46     | ![Structure](image) | | | 0.25 | 64 | 128 | 16 | 8 | 32 | 32 | 8 |
| Fluconazole | | | | 0.5 | 8 | >128 | 32 | 16 | 32 | 32 | 1 |

### Table 2. The antibacterial activity of 31 and 42.

| Compd. | Enterococcus faecalis UA257 | Staphylococcus aureus UA1758 | Staphylococcus epidermidis UF843 | Klebsiella pneumonia UF222 | Escherichia coli UA45 | ESBL-Producing Escherichia coli | Pseudomonas aeruginosa UA1024 | Acinetobacter baumannii UA1037 |
|--------|-----------------------------|-----------------------------|---------------------------------|--------------------------|----------------------|-------------------------------|-------------------------------|-------------------------------|
| 31     | >128 | 8 | 8 | >128 | >128 | >128 | >128 | >128 | >128 | >128 | >128 |
| 42     | >128 | 4 | 8 | >128 | >128 | >128 | >128 | >128 | >128 | >128 | >128 |
| Amikacin | n.t. | n.t. | n.t. | 2 | 8 | 3 | 3 | >256 | 6 | 32 |
| Cefoperazone | n.t. | n.t. | n.t. | 8 | 12 | 8 | 6 | 32 | 3 | 32 |
| Vancomycin | 1.5 | 0.75 | 2 | n.t. | n.t. | n.t. | n.t. | n.t. | n.t. | n.t. | n.t. |
| Erythromycin | 2 | >256 | 24 | n.t. | n.t. | n.t. | n.t. | n.t. | n.t. | n.t. | n.t. |

*a n.t. = Not tested.*
2.5. Antifungal Activity in Vivo

As compounds 31 and 42 demonstrated excellent antifungal activities \textit{in vitro} and were more potent than fluconazole, their \textit{in vivo} activities were investigated in the further studies. In these studies, a mouse model of systemic candidiasis was used. First, the toxicities of these compounds in the mice were explored. As the structures of the two compounds were similar, we only assessed the toxicity of compound 31. Each group of mice was given compound 31 by intragastric administration consecutively with dosages of 32, 16 or 8 mg/kg once a day. After five continuous days of drug administration, nothing abnormal was detected. Therefore, the further studies could be continued under these dosages. As shown in Figure 3, after a prolonged time of injection of ATCC 90028 (fluconazole-susceptible isolate), the mice in the control group died gradually, while the survival rates of mice treated with compound 31 or 42 increased.

\textbf{Figure 3.} Survival rate of mice after injection with \textit{C. albicans} ATCC 90028. FLZ stands for fluconazole and indicates a statistically significant difference compared to the control group. The four tested dosages of 31 and 42 were 32, 8, 2 and 0.5 mg/kg. The dosage of fluconazole was 0.5 mg/kg.

Compound 42 exhibited particularly excellent antifungal activity \textit{in vivo}; all mice treated with 32 mg/kg of 42 survived to day 10. However, compared with the reference drug fluconazole, the effects of the two compounds were weak, which was not consistent with the \textit{in vitro} assay results. This discrepancy may be due to the low hydrophilicity of compounds 31 and 42. In future studies, we will focus on improving the hydrophilicity of these compounds to enhance their \textit{in vivo} activity.

The effects of the two compounds in model mice treated with \textit{C. albicans} 64110 (fluconazole-resistant \textit{C. albicans} isolate) were also investigated, and the results are shown in Figure 4. Both compounds improved the survival rate of model mice, which indicated that they were effective against the fluconazole-resistant \textit{C. albicans} isolate \textit{in vivo}. Notably, 32 mg/kg of compound 31 kept most mice alive, displaying outstanding \textit{in vivo} activity toward the fluconazole-resistant \textit{C. albicans} isolate, which is promising regarding to solve clinical problems caused by fluconazole-resistant \textit{Candida} species.
Figure 4. Survival rate of mice after injection of *C. albicans* 64110. FLZ stands for fluconazole and indicates a statistically significant difference compared to the control group. The four tested dosages of 31 and 42 were 32, 8, 2 and 0.5 mg/kg.

The ED$_{50}$ of the compounds were also calculated and the results are presented in Table 3. Compound 31 was more effective toward fluconazole-resistant strain 64110 than fluconazole-susceptible isolate ATCC 90028, with ED$_{50}$ values of 2.693 and 21.653, respectively.

Table 3. The ED$_{50}$ (50% effective dose) values and 95% confidence intervals for the compounds.

| Compd. | ED$_{50}$ (mg/kg/day) | 95% Confidence Interval (mg/kg/day) |
|--------|-----------------------|-----------------------------------|
| 31     | 21.653                | 2.063–227.307                     |
|        | 2.693                 | 0.722–10.047                      |
| 42     | 6.812                 | 3.232–14.359                      |
|        | 6.944                 | 2.698–17.871                      |
| Fluconazole | 100% $^a$            |                                   |

$^a$ The survival rate of mice at 0.5 mg/kg dosage.

As for compound 42, the two ED$_{50}$ values were very similar, indicating similar activity against both strains.

3. Experimental Section

3.1. General Information

Reagents were purchased from Alfa Aesar (Shanghai, China), Acros Organics (Shanghai, China), Adamas-beta (Shanghai, China) and Shanghai Chemical Reagent Company (Shanghai, China) and were used without further purification. Analytical thin-layer chromatography (TLC) was performed using HSGF 254 plates (150–200-µm thickness, Yantai Huiyou Company, Yantai, China). Melting
points were measured without correction in capillary tubes on a SGW X-4 melting point apparatus. Nuclear magnetic resonance (NMR) spectra were acquired on a Bruker AVANCE 400 NMR instrument, using TMS as an internal standard. Chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane. Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q) or multiplet (m). Low- and high-resolution mass spectra (LRMS and HRMS) were acquired with electric ionization (EI) produced by a Finnigan MAT-95 spectrometer.

3.1.1. General Procedure for the Synthesis of 18o

The synthesis of 18o was performed according to a reported method [22].

3.1.2. General Procedure for the Synthesis of 18r

Compound 18r was synthesized according to a reported method [23].

3.1.3. General Procedure for the Synthesis of Bromoacetophenone Derivatives 19

A three-neck flask was charged with glacial acetic acid (35 mL) and the appropriate ketone (18, 0.02 mol). To this solution, bromine (1.1 eq) was added dropwise over 30 min at room temperature. The reaction mixture was irradiated at a power of 250 W for 5 h. The mixture was cooled, poured into 100 mL of H₂O, and extracted three times with CH₂Cl₂ (60 mL). The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The crude residue was purified by column chromatography (EtOAc/petroleum ether) to obtain the products. The data of the synthesized intermediates 19 is given in Table 4.

| Compd. | Chemical Structure | Appearance Property, Yield and 1H-NMR |
|--------|-------------------|-----------------------------------|
| 19a | ![Chemical Structure](image) | White solid; yield 72%. 1H-NMR (400 MHz, CDCl₃): δ 7.99 (d, J = 7.7 Hz, 2H), 7.62 (t, J = 7.4 Hz, 1H), 7.50 (t, J = 7.7 Hz, 2H), 4.47 (s, 2H). |
| 19k | ![Chemical Structure](image) | Yellow solid; yield 68.5%. 1H-NMR (400 MHz, CDCl₃): δ 7.87–7.82 (m, 1H), 7.81–7.76 (m, 1H), 7.33–7.27 (m, 1H), 4.39 (s, 2H). |
| 19m | ![Chemical Structure](image) | Yellow oil; yield 27%. 1H-NMR (400 MHz, CDCl₃): δ 8.22 (dd, J = 6.5, 2.0 Hz, 1H), 7.97–7.93 (m, 1H), 7.23 (dd, J = 15.7, 7.5 Hz, 1H), 4.41 (s, 2H), 2.59 (s, 1H). |
| 19n | ![Chemical Structure](image) | Yellow oil; yield 86%. 1H-NMR (500 MHz, acetone-d₆): δ 8.04–7.97 (m, 1H), 7.22–7.14 (m, 2H), 4.66 (d, J = 2.3 Hz, 2H). |
| 19o | ![Chemical Structure](image) | Yellow oil; yield 37.8%. 1H-NMR (400 MHz, CDCl₃): δ 7.94 (dd, J = 8.1, 2.3 Hz, 1H), 7.60–7.49 (m, 2H), 4.30 (s, 2H). |
| 19q | ![Chemical Structure](image) | Yellow oil; yield 75%. 1H-NMR (400 MHz, acetone-d₆): δ 8.74 (d, J = 4.6 Hz, 1H), 8.09–8.01 (m, 2H), 7.69 (m, 1H), 4.96 (s, 2H). |
| 19r | ![Chemical Structure](image) | Yellow oil; yield 47.7%. 1H-NMR (500 MHz, CDCl₃): δ 8.51 (d, J = 2.7 Hz, 1H), 8.11 (dd, J = 8.7, 4.7 Hz, 1H), 7.52 (m, 1H), 2.71 (s, 3H). |
| 19s | ![Chemical Structure](image) | Yellow solid; yield 46%. 1H-NMR (400 MHz, acetone-d₆): δ 7.92 (d, J = 0.9 Hz, 1H), 7.54–7.48 (m, 1H), 6.74 (dd, J = 3.6, 1.7 Hz, 1H), 4.53 (s, 2H). |
3.1.4. General Procedure for the Synthesis of 2-((1H-Imidazol-1-yl)-1-phenylethanone Derivatives 20

Bromoacetophenone derivatives 19 (1.0 mmol) and imidazole (2.0 mmol) were dissolved in dry tetrahydrofuran (10 mL) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography with dichloromethane/methanol (40:1) as the eluent to obtain the resulting intermediates. The data of the synthesized intermediates 20 is given in Table 5.

Table 5. The chemical structures, appearance, yields and 1H-NMR of intermediates 20.

| Compd. | Chemical Structure | Appearance Property, Yield and 1H-NMR |
|--------|-------------------|--------------------------------------|
| 20a    | ![Chemical Structure](image1) | Yellow solid; yield 60%. 1H-NMR (400 MHz, CDCl3): δ 7.99 (d, J = 7.8 Hz, 2H), 7.67 (t, J = 7.4 Hz, 1H), 7.62–7.50 (m, 3H), 7.16 (s, 1H), 6.97 (s, 1H), 5.43 (s, 2H). |
| 20b    | ![Chemical Structure](image2) | Yellow solid; yield 65%. 1H-NMR (400 MHz, CDCl3): δ 8.00 (d, J = 7.8 Hz, 2H), 7.70 (t, J = 7.4 Hz, 1H), 7.57 (t, J = 7.7 Hz, 2H), 7.46 (s, 1H), 5.37 (s, 2H). |
| 20c    | ![Chemical Structure](image3) | Yellow solid; yield 86%. 1H-NMR (400 MHz, CDCl3): δ 7.99 (dd, J = 8.4, 1.2 Hz, 2H), 7.68 (dd, J = 10.5, 4.4 Hz, 1H), 7.56 (d, J = 7.8 Hz, 2H), 7.44 (d, J = 0.9 Hz, 1H), 6.67 (s, 1H), 5.34 (s, 2H), 2.28 (d, J = 0.7 Hz, 3H). |
| 20d    | ![Chemical Structure](image4) | Yellow solid; yield 55%. 1H-NMR (400 MHz, CDCl3): δ 7.85 (d, J = 8.6 Hz, 2H), 7.69 (d, J = 8.6 Hz, 3H), 7.18 (s, 1H), 6.97 (s, 1H), 5.43 (s, 2H). |
| 20e    | ![Chemical Structure](image5) | Yellow solid; yield 45.6%. 1H-NMR (400 MHz, CDCl3): δ 7.92 (d, J = 8.3 Hz, 2H), 7.53 (t, J = 8.7 Hz, 3H), 7.16 (s, 1H), 6.96 (s, 1H), 5.40 (s, 2H). |
| 20f    | ![Chemical Structure](image6) | Yellow solid; yield 56%. 1H-NMR (400 MHz, CDCl3): δ 8.06-7.99 (m, 2H), 7.57 (s, 1H), 7.26–7.18 (m, 2H), 7.16 (s, 1H), 6.96 (s, 1H), 5.40 (s, 2H). |
| 20g    | ![Chemical Structure](image7) | Yellow solid; yield 53.6%. 1H-NMR (400 MHz, CDCl3): δ 7.88 (d, J = 8.2 Hz, 2H), 7.57 (s, 1H), 7.33 (d, J = 8.2 Hz, 2H), 7.15 (s, 1H), 6.96 (s, 1H), 5.40 (s, 2H), 2.45 (s, 3H). |
| 20h    | ![Chemical Structure](image8) | Yellow solid; yield 58.5%. 1H-NMR (400 MHz, CDCl3): δ 7.98 (d, J = 9.0 Hz, 2H), 7.58 (s, 1H), 7.16 (s, 1H), 7.02 (d, J = 9.0 Hz, 2H), 6.98 (s, 1H), 5.39 (s, 2H), 3.92 (s, 3H). |
| 20i    | ![Chemical Structure](image9) | Yellow solid; yield 55.3%. 1H-NMR (400 MHz, CDCl3): δ 8.08 (d, J = 8.1 Hz, 2H), 7.86 (d, J = 8.1 Hz, 2H), 7.56 (s, 1H), 7.17 (s, 1H), 6.96 (s, 1H), 5.44 (s, 2H). |
| 20j    | ![Chemical Structure](image10) | Yellow solid; yield 69.2%. 1H-NMR (400 MHz, CDCl3): δ 7.58 (d, J = 8.4 Hz, 1H), 7.53 (s, 1H), 7.51 (d, J = 1.6 Hz, 1H), 7.38 (dd, J = 8.4, 1.6 Hz, 1H), 7.11 (s, 1H), 6.94 (s, 1H), 5.35 (s, 2H). |
| 20k    | ![Chemical Structure](image11) | Yellow solid; yield 56%. 1H-NMR (400 MHz, CDCl3): δ 7.88-7.80 (m, 1H), 7.80–7.74 (m, 1H), 7.58 (s, 1H), 7.35 (dd, J = 17.0, 8.6 Hz, 1H), 7.17 (s, 1H), 6.96 (s, 1H), 5.39 (s, 2H). |
| 20l    | ![Chemical Structure](image12) | Yellow solid; yield 47.2%. 1H-NMR (400 MHz, CDCl3): δ 8.08 (dd, J = 6.9, 1.9 Hz, 1H), 7.95–7.87 (m, 1H), 7.76 (s, 1H), 7.32 (t, J = 8.5 Hz, 1H), 7.19 (s, 1H), 6.98 (s, 1H), 5.46 (s, 2H). |
| 20m    | ![Chemical Structure](image13) | Yellow solid; yield 55.5%. 1H-NMR (400 MHz, CDCl3): δ 8.22 (dd, J = 6.4, 2.1 Hz, 1H), 7.96–7.92 (m, 1H), 7.56 (s, 1H), 7.28 (d, J = 8.1 Hz, 1H), 7.16 (s, 1H), 6.95 (s, 1H), 5.39 (s, 2H). |
| 20n    | ![Chemical Structure](image14) | Yellow solid; yield 17.4%. 1H-NMR (500 MHz, acetone-d6): δ 8.10-8.06 (m, 1H), 7.56 (s, 1H), 7.33–7.20 (m, 2H), 7.10 (d, J = 1.0 Hz, 1H), 6.96 (s, 1H), 5.61 (d, J = 3.4 Hz, 2H). |
| 20o    | ![Chemical Structure](image15) | Yellow solid; yield 58.3%. 1H-NMR (400 MHz, CDCl3): δ 7.93 (dd, J = 8.0, 2.4 Hz, 1H), 7.57 (s, 1H), 7.64–7.59 (m, 1H), 7.44 (dd, J = 8.4, 5.2 Hz, 1H), 7.11 (s, 1H), 6.98 (s, 1H), 5.15 (s, 2H). |
| 20p    | ![Chemical Structure](image16) | Yellow solid; yield 75%. 1H-NMR (400 MHz, acetone-d6): δ 8.18 (d, J = 8.3 Hz, 2H), 7.89 (d, J = 8.3 Hz, 2H), 7.78 (d, J = 7.7 Hz, 2H), 7.71 (s, 1H), 7.52 (d, J = 7.7 Hz, 2H), 7.46 (t, J = 7.3 Hz, 1H), 7.18 (s, 1H), 7.03 (s, 1H), 5.85 (s, 2H). |
3.1.5. General Procedure for the Synthesis of 21–44 and 46

To a 25-mL round-bottom flask charged with the appropriate 2-(1H-imidazol-1-yl)-1-phenyl-ethanone derivative 20 (1.6 mmol), (E)-3-(4-nitrophenyl) acrylaldehyde (1.2 eq) and toluene (4 mL) was added piperidine (55 μL) and glacial acetic acid (12 μL). The flask was evacuated, filled with nitrogen and heated to 75 °C for 4 h. The reaction mixture was cooled to room temperature and the solvent was removed. The reaction mixture was purified by silica gel column chromatography with triethylamine/petroleum ether/ethyl acetate (0.15:2:1, v/v/v) as the eluent to obtain the crude product. The crude product was washed with petroleum ether/ethyl acetate (70:1, v/v) to obtain the final product.

3.1.6. Synthesis of 45

A mixture of 31 (300 mg, 0.76 mmol) and sodium borohydride (165 mg) in absolute methanol (15 mL) was stirred at room temperature for 2 h. After removing the solvent, the residue was purified by flash column chromatography on silica gel, eluted with triethylamine/petroleum ether/ethyl acetate (0.15:2:1, v/v/v), to afford 45 (200 mg, 66%) as yellow oil. The data of target compounds 21–46 are given in Table 6.

3.2. Biological Assays

3.2.1. Antifungal Susceptibility Tests

The prepared compounds and fucconazole (Pfizer Pharmaceuticals, Shanghai, China) were dissolved in DMSO to prepare primary stocks.

The stock was then gradually diluted to prepare secondary stocks with different concentrations. Finally, the working concentrations of the derivatives were obtained by adding the appropriate amount of the secondary DMSO stocks to RPMI 1640 medium. The amount of DMSO in working solutions did not exceed 1%. Antifungal susceptibility tests were performed according to the standard guidelines described in the Clinical and Laboratory Standards Institute document M27-A3, and the microdilution reference method was used. Next, 100 μL of RPMI 1640 medium containing the desired concentrations (128 mg/L to 0.5 mg/L) of the appropriate compound was added to each well of a 96-well plate.
Table 6. The chemical structures, properties, yields, $^1$H-NMR and HRMS of target compounds 21–46.

| Compd. | Chemical Structure | Properties | Yield, $^1$H-NMR and HRMS |
|--------|-------------------|------------|---------------------------|
| 21     | ![Chemical structure](image) | Yellow solid, mp 150–151 °C | Yield 20.8%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 8.24 (d, $J = 8.8$ Hz, 2H), 7.89 (s, 1H), 7.81 (d, $J = 7.6$ Hz, 2H), 7.72 (d, $J = 8.8$ Hz, 2H), 7.67 (d, $J = 7.1$ Hz, 1H), 7.57 (t, $J = 7.7$ Hz, 2H), 7.48 (d, $J = 11.1$ Hz, 1H), 7.35 (s, 1H), 7.22 (s, 1H), 6.98 (dd, $J = 15.5, 11.1$ Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{15}$N$_3$O$_3$ (M$^+$) 345.1106, found: 345.1113, 223.0860 (100%). |
| 22     | ![Chemical structure](image) | Yellow solid, mp 162–165 °C | Yield 9.7%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 8.01 (d, $J = 8.0$ Hz, 1H), 7.89–7.73 (m, 5H), 7.68–7.56 (m, 2H), 7.55 (t, $J = 7.6$ Hz, 2H), 7.49 (d, $J = 11.1$ Hz, 1H), 7.32 (s, 1H), 7.10 (s, 1H), 6.91 (dd, $J = 15.4, 11.1$ Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{15}$N$_3$O$_3$ (M$^+$) 345.1113, found: 345.1110, 105.0341 (100%). |
| 23     | ![Chemical structure](image) | Yellow solid, mp 161–163 °C | Yield 10%. $^1$H-NMR (400 MHz, methanol-$d_4$): $\delta$ 7.84 (s, 1H), 7.79 (d, $J = 7.3$ Hz, 2H), 7.67 (t, $J = 7.4$ Hz, 1H), 7.60–7.49 (m, 4H), 7.47 (d, $J = 11.1$ Hz, 1H), 7.31 (s, 1H), 7.26 (d, $J = 15.5$ Hz, 1H), 7.21 (s, 1H), 7.12 (t, $J = 8.7$ Hz, 2H), 6.72 (dd, $J = 15.4, 11.1$ Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{15}$F$_3$N$_2$O$_2$ (M$^+$) 318.1168, found: 318.1164, 223.0870 (100%). |
| 24     | ![Chemical structure](image) | Yellow solid, mp 117–120 °C | Yield 9.5%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 7.77 (d, $J = 7.6$ Hz, 2H), 7.72 (s, 1H), 7.66 (t, $J = 7.4$ Hz, 1H), 7.62–7.46 (m, 6H), 7.39 (d, $J = 11.1$ Hz, 1H), 7.34–7.25 (m, 2H), 7.11 (s, 1H), 6.90 (dd, $J = 15.5, 11.1$ Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{15}$Br$_3$N$_2$O (M$^+$) 378.0368, found: 378.0360, 223.0864 (100%). |
| 25     | ![Chemical structure](image) | Yellow solid, mp 133–137 °C | Yield 11.4%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 7.73 (d, $J = 7.1$ Hz, 2H), 7.67–7.59 (m, 2H), 7.53 (t, $J = 7.5$ Hz, 2H), 7.38 (dd, $J = 15.6, 10.1$ Hz, 3H), 7.19 (d, $J = 18.7$ Hz, 2H), 7.10 (s, 1H), 6.72 (d, $J = 8.9$ Hz, 2H), 6.56 (dd, $J = 15.3, 11.3$ Hz, 1H), 2.83 (d, $J = 13.3$ Hz, 3H). HRMS (EI) calculated for C$_{21}$H$_{18}$N$_2$O$_2$ (M$^+$) 314.1419, found: 314.1411, 343.1678 (100%). |
| 26     | ![Chemical structure](image) | Yellow solid, mp 190–192 °C | Yield 9.8%. $^1$H-NMR (400 MHz, methanol-$d_4$): $\delta$ 7.87 (s, 1H), 7.81 (d, $J = 7.4$ Hz, 2H), 7.68 (t, $J = 7.5$ Hz, 1H), 7.63–7.52 (m, 5H), 7.49 (d, $J = 11.1$ Hz, 1H), 7.34 (d, $J = 13.2$ Hz, 2H), 7.20 (s, 1H), 6.84 (dd, $J = 15.5, 11.1$ Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{13}$Cl$_2$N$_2$O (M$^+$) 368.0483, found: 368.0474, 223.0856 (100%). |
| 27     | ![Chemical structure](image) | Yellow solid, mp 181–183 °C | Yield 28%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 7.83 (d, $J = 7.7$ Hz, 3H), 7.70 (t, $J = 7.4$ Hz, 1H), 7.65 (d, $J = 11.1$ Hz, 1H), 7.63–7.55 (m, 6H), 7.42 (d, $J = 15.5$ Hz, 1H), 7.02 (dd, $J = 15.5, 11.1$ Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{13}$Br$_3$Cl$_2$N$_2$O (M$^+$) 445.9586, found: 445.9586, 105.0319 (100%). |
| 28     | ![Chemical structure](image) | Yellow solid, mp 172–173 °C | Yield 11.3%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 7.76 (d, $J = 7.0$ Hz, 2H), 7.65 (t, $J = 7.4$ Hz, 1H), 7.59–7.47 (m, 7H), 7.28 (dd, $J = 15.9, 13.3$ Hz, 2H), 7.01–6.92 (m, 2H), 2.20 (s, 3H). HRMS (EI) calculated for C$_{21}$H$_{13}$Br$_2$N$_2$O (M$^+$) 392.0524, found: 392.0522, 237.1022 (100%). |
| 29     | ![Chemical structure](image) | Yellow solid, mp 162–164 °C | Yield 35.4%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 7.77–7.68 (m, 5H), 7.58 (d, $J = 8.5$ Hz, 2H), 7.49 (d, $J = 8.5$ Hz, 2H), 7.44 (d, $J = 11.1$ Hz, 1H), 7.34 (d, $J = 15.5$ Hz, 1H), 7.27 (s, 1H), 7.11 (s, 1H), 6.90 (dd, $J = 15.5, 11.1$ Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{14}$Br$_2$N$_2$O (M$^+$) 455.9473, found: 455.9465, 300.9948 (100%). |
| Compd. | Chemical Structure | Properties | Yield, $^1$H-NMR and HRMS |
|-------|-------------------|------------|--------------------------|
| 30    | ![Chemical Structure](image30) | Yellow solid, mp 150–153 °C | Yield 25.8%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 7.78 (d, $J = 8.5$ Hz, 2H), 7.71 (s, 1H), 7.58 (d, $J = 7.1$ Hz, 4H), 7.49 (d, $J = 8.5$ Hz, 2H), 7.43 (d, $J = 11.1$ Hz, 1H), 7.33 (d, $J = 15.6$ Hz, 1H), 7.27 (s, 1H), 7.11 (s, 1H), 6.90 (dd, $J = 15.5$, 11.1 Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{13}$BrClF$_2$N$_2$O (M$^+$) 429.9883, found: 429.9884, 275.0367 (100%) |
| 31    | ![Chemical Structure](image31) | Yellow solid, mp 115–116 °C | Yield 23.4%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 7.86 (dd, $J = 8.7$, 5.5 Hz, 2H), 7.72 (s, 1H), 7.58 (d, $J = 8.5$ Hz, 2H), 7.49 (d, $J = 8.5$ Hz, 2H), 7.41 (d, $J = 11.1$ Hz, 1H), 7.35–7.24 (m, 4H), 7.11 (s, 1H), 6.90 (dd, $J = 15.5$, 11.1 Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{13}$BrF$_2$N$_2$O (M$^+$) 396.0274, found: 396.0272, 241.0771 (100%) |
| 32    | ![Chemical Structure](image32) | Yellow solid, mp 153–155 °C | Yield 9.5%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 7.77 (d, $J = 8.9$ Hz, 2H), 7.71 (s, 1H), 7.58 (d, $J = 8.5$ Hz, 2H), 7.48 (d, $J = 8.5$ Hz, 2H), 7.36–7.24 (m, 3H), 7.10 (s, 1H), 7.05 (d, $J = 8.9$ Hz, 2H), 6.90 (dd, $J = 15.5$, 11.2 Hz, 1H), 2.42 (d, $J = 14.3$ Hz, 3H). HRMS (EI) calculated for C$_{21}$H$_{14}$BrClF$_2$N$_2$O (M$^+$) 392.0524, found: 392.0524, 237.1025 (100%) |
| 33    | ![Chemical Structure](image33) | Yellow solid, mp 166–167 °C | Yield 9.5%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 7.77 (d, $J = 8.9$ Hz, 2H), 7.71 (s, 1H), 7.58 (d, $J = 8.5$ Hz, 2H), 7.48 (d, $J = 8.5$ Hz, 2H), 7.36–7.24 (m, 3H), 7.10 (s, 1H), 7.05 (d, $J = 8.9$ Hz, 2H), 6.90 (dd, $J = 15.5$, 11.1 Hz, 1H), 2.42 (d, $J = 14.3$ Hz, 3H). HRMS (EI) calculated for C$_{21}$H$_{14}$BrClF$_2$N$_2$O (M$^+$) 408.0473, found: 408.0475, 253.0972 (100%) |
| 34    | ![Chemical Structure](image34) | Yellow solid, mp 174–178 °C | Yield 55.3%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 7.95 (q, $J = 8.6$ Hz, 4H), 7.72 (s, 1H), 7.58 (d, $J = 8.5$ Hz, 2H), 7.48 (d, $J = 9.5$, 7.8 Hz, 3H), 7.34 (d, $J = 15.5$ Hz, 1H), 7.28 (s, 1H), 7.11 (s, 1H), 6.91 (dd, $J = 15.5$, 11.1 Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{13}$BrClF$_2$N$_2$O (M$^+$) 403.0320, found: 403.0319, 248.0824 (100%) |
| 35    | ![Chemical Structure](image35) | Yellow solid, mp 197–199 °C | Yield 31%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 7.66 (d, $J = 12.3$ Hz, 3H), 7.57 (d, $J = 8.2$ Hz, 3H), 7.47 (d, $J = 8.1$ Hz, 2H), 7.35 (dd, $J = 21.5$, 13.3 Hz, 1H), 7.25 (s, 1H), 7.13 (s, 1H), 6.87 (dd, $J = 15.1$, 11.3 Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{13}$BrClF$_2$N$_2$O (M$^+$) 445.9588, found: 445.9588, 291.0083 (100%) |
| 36    | ![Chemical Structure](image36) | Yellow solid, mp 132–133 °C | Yield 74.9%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 7.78–7.70 (m, 2H), 7.65 (s, 1H), 7.58 (d, $J = 8.4$ Hz, 2H), 7.50 (t, $J = 8.9$ Hz, 4H), 7.34 (d, $J = 15.5$ Hz, 1H), 7.28 (s, 1H), 7.11 (s, 1H), 6.90 (dd, $J = 15.5$, 11.1 Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{13}$BrClF$_2$N$_2$O (M$^+$) 414.0179, found: 414.0177, 259.0675 (100%) |
| 37    | ![Chemical Structure](image37) | Yellow solid, mp 111–112 °C | Yield 52.1%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 7.91 (dd, $J = 7.2$, 2.1 Hz, 1H), 7.80–7.77 (m, 1H), 7.73 (s, 1H), 7.58 (d, $J = 8.5$ Hz, 2H), 7.49 (dd, $J = 13.0$, 4.7 Hz, 4H), 7.34 (d, $J = 15.6$ Hz, 1H), 7.28 (s, 1H), 7.11 (s, 1H), 6.90 (dd, $J = 15.5$, 11.1 Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{13}$BrClF$_2$N$_2$O (M$^+$) 429.9884, found: 429.9883, 275.0367 (100%) |
| 38    | ![Chemical Structure](image38) | Yellow solid, mp 133–134 °C | Yield 89%. $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ 8.04 (dd, $J = 6.7$, 2.1 Hz, 1H), 7.84–7.80 (m, 1H), 7.73 (s, 1H), 7.58 (d, $J = 8.5$ Hz, 2H), 7.49 (d, $J = 8.8$ Hz, 3H), 7.44 (d, $J = 8.6$ Hz, 1H), 7.34 (d, $J = 15.6$ Hz, 1H), 7.29 (s, 1H), 7.11 (s, 1H), 6.90 (dd, $J = 15.5$, 11.1 Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{13}$BrClF$_2$N$_2$O (M$^+$) 473.9379, found: 473.9381, 318.9905 (100%) |
| Compd. | Chemical Structure | Properties | Yield, $^1$H-NMR and HRMS |
|--------|-------------------|------------|--------------------------|
| 39     | ![Chemical Structure](image1) | Yellow solid, mp 148–150 °C | Yield 55.1%. $^1$H-NMR (500 MHz, acetone-$d_6$): δ 7.79–7.72 (m, 1H), 6.79 (s, 1H), 7.59 (d, $J$ = 8.5 Hz, 2H), 7.48 (dd, $J$ = 13.8, 9.9 Hz, 3H), 7.34 (d, $J$ = 15.5 Hz, 1H), 7.25–7.17 (m, 3H), 7.11 (s, 1H), 6.90 (dd, $J$ = 15.5, 11.1 Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{13}$BrF$_2$N$_2$O (M$^+$) 414.0179, found: 414.0166, 259.0647 (100%). |
| 40     | ![Chemical Structure](image2) | Yellow solid, mp 183–184 °C | Yield 52.3%. $^1$H-NMR (500 MHz, acetone-$d_6$): δ 8.12 (dd, $J$ = 8.6, 2.5 Hz, 1H), 7.88 (dd, $J$ = 8.5, 5.4 Hz, 1H), 7.80–7.83 (m, 1H), 7.65 (s, 1H), 7.56 (d, $J$ = 8.5 Hz, 2H), 7.45 (d, $J$ = 8.5 Hz, 2H), 7.39 (d, $J$ = 11.0 Hz, 1H), 7.26–7.21 (m, 2H), 7.12 (s, 1H), 6.83 (dd, $J$ = 15.5, 11.1 Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{13}$BrF$_2$N$_2$O (M$^+$) 441.0124, found: 441.0130, 288.9987 (100%). |
| 41     | ![Chemical Structure](image3) | Yellow solid, mp 167–168 °C | Yield 65.5%. $^1$H-NMR (400 MHz, acetone-$d_6$): δ 7.90 (d, $J$ = 8.3 Hz, 2H), 7.85 (d, $J$ = 8.3 Hz, 2H), 7.78 (d, $J$ = 6.7 Hz, 4H), 7.66–7.50 (m, 5H), 7.46 (d, $J$ = 7.3 Hz, 1H), 7.42 (d, $J$ = 8.6 Hz, 1H), 7.32 (s, 1H), 7.12 (s, 1H), 6.98 (dd, $J$ = 15.5, 11.1 Hz, 1H). HRMS (EI) calculated for C$_{19}$H$_{12}$BrN$_2$O (M$^+$) 431.0659, found: 431.0648, 299.1186 (100%). |
| 42     | ![Chemical Structure](image4) | Yellow solid, mp 114–116 °C | Yield 63.5%. $^1$H-NMR (500 MHz, acetone-$d_6$): δ 8.66 (d, $J$ = 2.8 Hz, 1H), 8.13 (d, $J$ = 11.2 Hz, 1H), 8.05 (t, $J$ = 7.1 Hz, 1H), 7.94 (d, $J$ = 7.8 Hz, 1H), 7.69–7.89 (m, 1H), 7.69 (s, 1H), 7.62 (d, $J$ = 8.5 Hz, 2H), 7.52 (d, $J$ = 8.5 Hz, 2H), 7.36 (d, $J$ = 15.6 Hz, 1H), 7.23 (s, 1H), 7.10 (s, 1H), 6.84 (dd, $J$ = 15.6, 11.2 Hz, 1H). HRMS (EI) calculated for C$_{19}$H$_{12}$BrN$_2$O (M$^+$) 389.0320, found: 379.0318, 224.0823 (100%). |
| 43     | ![Chemical Structure](image5) | Yellow solid, mp 156–157 °C | Yield 4.3%. $^1$H-NMR (500 MHz, acetone-$d_6$): δ 8.66 (d, $J$ = 2.8 Hz, 1H), 8.16–8.08 (m, 2H), 7.93–7.89 (m, 1H), 7.69 (s, 1H), 7.62 (d, $J$ = 8.5 Hz, 2H), 7.52 (d, $J$ = 8.5 Hz, 2H), 7.36 (d, $J$ = 15.6 Hz, 1H), 7.24 (s, 1H), 7.13 (s, 1H), 6.88 (dd, $J$ = 15.6, 11.2 Hz, 1H). HRMS (EI) calculated for C$_{19}$H$_{12}$BrN$_2$O (M$^+$) 397.0226, found: 397.0227, 242.0732 (100%). |
| 44     | ![Chemical Structure](image6) | Yellow solid, mp 180–182 °C | Yield 21.1%. $^1$H-NMR (400 MHz, acetone-$d_6$): δ 7.90 (d, $J$ = 0.9 Hz, 1H), 7.78 (d, $J$ = 11.2 Hz, 1H), 7.74 (s, 1H), 7.59 (d, $J$ = 8.5 Hz, 1H), 7.49 (d, $J$ = 8.5 Hz, 2H), 7.40 (d, $J$ = 15.6 Hz, 1H), 7.29 (s, 1H), 7.17 (s, 1H), 6.78 (dd, $J$ = 15.6, 11.2 Hz, 1H), 6.65 (dd, $J$ = 3.6, 1.6 Hz, 1H), 6.57 (d, $J$ = 3.5 Hz, 1H). HRMS (EI) calculated for C$_{19}$H$_{12}$BrN$_2$O$_2$ (M$^+$) 368.0160, found: 368.0157, 213.0664 (100%). |
| 45     | ![Chemical Structure](image7) | Yellow oil | Yield 66%. $^1$H-NMR (400 MHz, acetone-$d_6$): δ 7.50 (d, $J$ = 8.5 Hz, 2H), 7.40 (s, 1H), 7.36–7.32 (m, 4H), 7.04 (t, $J$ = 8.8 Hz, 2H), 6.99 (s, 1H), 6.94 (s, 1H), 6.89 (d, $J$ = 15.7 Hz, 1H), 6.81 (d, $J$ = 11.0 Hz, 1H), 6.57 (dd, $J$ = 15.7, 10.9 Hz, 1H), 5.64 (d, $J$ = 8.2 Hz, 1H). HRMS (EI) calculated for C$_{20}$H$_{16}$BrFN$_2$O (M$^+$) 398.0430, found: 398.0424, 243.0927 (100%). |
| 46     | ![Chemical Structure](image8) | Yellow oil | Yield 20.1%. $^1$H-NMR (500 MHz, acetone-$d_6$): δ 7.76 (dd, $J$ = 8.5, 5.6 Hz, 2H), 7.48 (d, $J$ = 8.2 Hz, 3H), 7.26 (t, $J$ = 8.7 Hz, 2H), 7.17 (d, $J$ = 8.1 Hz, 2H), 7.03 (d, $J$ = 10.1 Hz, 2H), 6.74 (t, $J$ = 7.4 Hz, 1H), 2.86 (t, $J$ = 7.3 Hz, 2H), 2.58 (q, $J$ = 7.4 Hz, 2H). HRMS (EI) calculated for C$_{20}$H$_{16}$BrFN$_2$O (M$^+$) 398.0430, found: 398.0433, 123.0243 (100%). |
Eight strains of Candida spp. were used in the assay, including the quality control Candida albicans ATCC 90028 and Candida krusei ATCC 6528 isolates. They were cultured in solid Yeast Extract Peptone Dextrose (YPD) medium at 37 °C in a humidified atmosphere of 5% CO₂ in air. The cells were dissolved in normal saline at a density of 5 × 10⁶ CFU/mL. Then, the solution was diluted 1000 times with RPMI 1640 medium, and 100 μL of diluted solution was added to the 96-well plate containing the compounds. After incubation for 48 h at 37 °C, the MIC was read as the lowest concentration that produced a prominent decrease in growth (inhibition ≥ 80%) compared to the control cells (without compound).

3.2.2. Antibacterial Susceptibility Tests

The microdilution reference method was performed according to the standard guidelines described in the Clinical and Laboratory Standards Institute document M07-A9 for the antibacterial susceptibility assays. The desired working concentrations of the derivatives 31, 42, and four positive drugs (amikacin, cefoperazone, vancomycin, and erythromycin) were obtained by adding the secondary DMSO stocks to broth culture, as described for the antifungal susceptibility assays. Then, 100 μL of broth culture containing the appropriate concentrations (128 mg/L to 0.5 mg/L) of each compound was added to each well of a 96-well plate.

Eight strains of bacteria were used: Gram-positive bacterial isolates Staphylococcus aureus UA1758, Staphylococcus epidermidis UF843, and Enterococcus faecalis UA257, and Gram-negative bacterial isolates Klebsiella pneumonia UF222, Escherichia coli UA45, ESBL-producing Escherichia coli, Acinetobacter baumannii UA1037, and Pseudomonas aeruginosa UA1024. After culture in a blood plate at 37 °C in a humidified atmosphere of 5% CO₂ in air, cells were suspended in normal saline at a density of 2 × 10⁸ CFU/mL. Then, the solution was diluted 1000 times with culture broth, and 100 μL of diluted solution was added to the 96-well plate containing compounds. After incubation for 24 h at 37 °C, the MIC was read as the lowest concentration that produced a prominent decrease in growth (100% inhibition) compared with the control cells (without compound).

3.2.3. Mice Toxicity Assays

Sixteen mice (equal numbers of males and females) were selected to test the toxicity of compound 31 in mice. They were randomly grouped into four groups (four mice in each group): 32 mg/kg group, 16 mg/kg group, 8 mg/kg group and control group. Each group of mice was given compound 31 by intragastric administration consecutively for 5 days with dosages of 32, 16 and 8 mg/kg once a day. The mice in the control group were given 0.5% sodium carboxymethyl cellulose solution at 20 mL/kg. The survival of the mice was recorded.

3.2.4. In Vivo Antifungal Activity

To establish a systemic fungal infection model, 160 Kunming mice, half males and half females, were selected, with weights ranging from 18 to 22 g and that had passed the quarantine inspection. They were divided into four groups according to their weight: compound 31 strain No. 1 group (I), compound 31 strain No. 2 group (II), compound 42 strain No. 1 group (III) and compound 42 strain
No. 2 group (IV). Each group contained equal numbers of males and females. The 40 mice in group I containing half males and half females were divided into four dose groups and a control group, with 8 mice in each group, containing four of each sex. The mice in each dose group were given a different dosage (0.5, 2, 8 or 32 mg/kg) of compound 31 by intragastric administration, and the mice in the control group were given 0.5% sodium carboxymethyl cellulose solution at 20 mL/kg. A concentration of \(2 \times 10^6\) CFU/mL of strain No. 1 was given by tail vein injection approximately 0.5 h after intragastric administration of the compound. Drugs were administered to mice once a day for 10 consecutive days. The survival rate and ED\(_{50}\) were calculated for each group. The remaining groups (II, III and IV) were given the relevant drugs and strains according to the group designations presented above. The test method was similar to what is described above. Testing of fluconazole (positive control) was performed according to the method similar to that described above.

Result processing: the ED\(_{50}\) and 95% confidence limit were calculated for each group using the regular Bliss method, and a graph of the survival curve was plotted based on the relationship between the survival rates of the mice over time.

4. Conclusions

In conclusion, we observed inhibitory activity of compound 24 containing an imidazole moiety and a 2,4-dienone motif toward several fungi. Based on this, a total of 26 derivatives were designed and synthesized in three steps. The prepared compounds were tested using \textit{in vitro} antifungal activity assays, and several compounds exhibited improved antifungal activities compared to the lead compound. Among these compounds, compounds 31 and 42 exhibited strong inhibitory effects toward \textit{Candida} species. In particular, the two derivatives exhibited potent antifungal activities toward the fluconazole-resistant isolate \textit{C. albicans} 64110, with both having MIC values of 8 µg/mL. In addition, they displayed obvious effects against two Gram-positive bacteria, \textit{Staphylococcus epidermidis} UF843 and \textit{Staphylococcus aureus} UA1758. The results of animal experiments indicated that both compounds could improve the survival rate of model mice treated with ATCC 90028 (fluconazole-susceptible isolate). More importantly, the two compounds exhibited outstanding effects toward a fluconazole-resistant \textit{C. albicans} isolate \textit{in vivo}, which is promising with regard to the clinical problem posed by fluconazole-resistant \textit{Candida} species.

Acknowledgments

Financial support of this research provided by the National Natural Science Foundation of China (Grants 21222211, 21372001, 91313303), the Program for New Century Excellent Talents in University (Grant NCET-12-0853), the Fundamental Research Funds for the Central Universities is gratefully acknowledged and the Scientific Research Key Project of Shanghai Municipal Health Bureau.

Author Contributions

J.L., M.X. and F.M. designed research; C.L., C.S., J.L., B.W. and Y.X. performed research and analyzed the data; F.M., C.L. and J.Z. wrote the paper. All authors read and approved the final manuscript.
Conflicts of Interest

The authors declare no conflict of interest.

References

1. Danishuddin, M.; Kaushal, L.; Hassan Baig, M.; Khan, A.U. AMDD: Antimicrobial drug database. Genomics Proteomics Bioinform. 2012, 10, 360–363.
2. Lafleur, M.D.; Sun, L.; Lister, I.; Keating, J.; Nantel, A.; Long, L.; Ghannoum, M.; North, J.; Lee, R.E.; Coleman, K.; et al. Potentiation of Azole Antifungals by 2-Adamantanamine. Antimicrob. Agents Chemother. 2013, 57, 3585–3591.
3. Lloyd, D.H. Alternatives to conventional antimicrobial drugs: A review of future prospects. Vet. Dermatol. 2012, 23, 299–304.
4. Wang, X.L.; Zhou, C.H; Geng, R.X. Advance in the research of antimicrobial drugs with sulfamide group. Chin. J. New Drug. 2010, 19, 2050–2059.
5. MTünçbilek, M.; Kiper, T.; Altanlar, N. Synthesis and in vitro antimicrobial activity of some novel substituted benzimidazole derivatives having potent activity against MRSA. Eur. J. Med. Chem. 2009, 44, 1024–1033.
6. Sharma, D.; Narasimhan, B.; Kumar, P.; Jalbout, A. Synthesis and QSAR evaluation of 2-(substituted phenyl)-1H-benzimidazoles and [2-(substituted phenyl)-benzimidazol-1-yl]-pyridin-3-yl-methanones. Eur. J. Med. Chem. 2009, 44, 1119–1127.
7. Sharma, S.; Gangal, S.; Rauf, A. Convenient one-pot synthesis of novel 2-substituted benzimidazoles tetrahydrobenzimidazoles and imidazoles and evaluation of their in vitro antibacterial and antifungal activities. Eur. J. Med. Chem. 2009, 44, 1751–1757.
8. Peng, X.M.; Cai, G.X.; Zhou, C.H. Recent developments in azole compounds as antibacterial and antifungal agents. Curr. Top. Med. Chem. 2013, 13, 1963–2010.
9. McCarthy, K.M.; Morgan, J.; Wannemuehler, K.A.; Mirza, S.A.; Gould, S.M.; Mhlongo, N.; Moeng, P.; Maloba, B.R.; Crewe-Brown, H.H.; Brandt, M.E.; et al. Population-based surveillance for cryptococcosis in an antiretroviral-naive South African province with a high HIV seroprevalence. AIDS 2006, 20, 2199–2206.
10. Boiani, M.; Gonzalez, M. Imidazole and benzimidazole derivatives as chemotherapeutic agents. Mini-Rev. Med. Chem. 2005, 5, 409–424.
11. Dhainaut, A.; Tizot, A.; Raimbaud, E.; Lockhart, B.; Lestage, P.; Goldstein, S. Synthesis, structure, and neuroprotective properties of novel imidazolyl nitrones. J. Med. Chem. 2000, 43, 2165–2175.
12. Rani, N.; Sharma, A.; Gupta, G.K.; Singh, R. Imidazoles as potential antifungal agents: A review. Mini-Rev. Med. Chem. 2013, 13, 1626–1655.
13. Khan, Z.K.; Jain, P. Antifungal agents and immunomodulators in systemic mycoses. Indian J. Chest Dis. Allied Sci. 2000, 42, 345–355.
14. Sud, I.J.; Chou, D.L.; Feingold, D.S. Effect of free fatty acids on liposome susceptibility to imidazole antifungals. Antimicrob. Agents Chemother. 1979, 16, 660–663.
15. Mahmoudabadi, A.; Drucker, D. Effect of Amphotericin B, Nystatin and Miconazole on the polar lipids of *C. albicans* and *C. Dublieniensis*. *Indian J. Pharmacol.* **2006**, *38*, 423–426.
16. Bossche, H.V.; Engelen, M.; Rochette, F. Antifungal agents of use in animal health—Chemical, biochemical and pharmacological aspects. *J. Vet. Pharmacol. Ther.* **2003**, *26*, 5–29.
17. Niwano, Y.; Tabuchi, T.; Kanai, K.; Hamaguchi, H.; Uchida, K.; Yamaguchi, H. Short-term topical therapy of experimental Tinea pedis in guinea pigs with Lanoconazole, a new imidazole antymycotic agent. *Antimicrob. Agents Chemother.* **1995**, *39*, 2353–2355.
18. Niwano, Y.; Ohmi, T.; Seo, A.; Kodama, H.; Koga, H.; Sakai, A. Lanoconazole and its related optically active compound NND-502: Novel antifungal imidazoles with a ketene dithioacetal structure. *Curr. Med. Chem. Anti-Infect. Agents* **2003**, *2*, 147–160.
19. Jones, B.M.; Geary, I.; Lee, M.E.; Duerden, B.I. Comparison of the *in vitro* activities of fenticonazole, other imidazoles, metronidazole, and tetracycline against organisms associated with bacterial vaginosis and skin infections. *Antimicrob. Agents Chemother.* **1989**, *33*, 970–972.
20. Lakshmanan, B.; Mazumder, P.M.; Sasmal, D.; Ganguly, S. Biologically active azoles: Synthesis, characterization and antimicrobial activity of some 1-substituted imidazoles. *Pharm. Lett.* **2010**, *2*, 82–89.
21. Babu, K.S.; Li, X.-C.; Jacob, M.R.; Zhang, Q.; Khan, S.I.; Ferreira, D.; Clark, A.M. Synthesis, antifungal activity, and structure-activity relationships of coruscanone A analogues. *J. Med. Chem.* **2006**, *49*, 7877–7886.
22. Abbot, S.C.; Boice, G.N.; Buettelmann, B.; Goldstein, D.M.; Gong, L.; Hogg, J.H.; Iyer, P.; McCaleb, K.L.; Tan, Y.-C. Dihydroquinone and Dihydronaphthridine Inhibitors of JNK. U.S. Patent 20080287458 A1, 20 November 2008.
23. Almeida, L.; Ioannidis, S.; Lamb, M.; Su, M. Pyrazolyl-Amino-Substituted Pyrazines and Their Use for the Treatment of Cancer. WO2008117050 A1, 2 October 2008.

*Sample Availability:* Samples of the compounds are available from the authors.