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# Chalcone-Benzotriazole Conjugates as New Potential Antimicrobial Agents: Design, Synthesis, Biological Evaluation and Synergism with Clinical Drugs

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A series of chalcone-benzotriazole conjugates as new potential antimicrobial agents were synthesized and characterized by $^{1}$H NMR, $^{13}$C NMR, IR and HRMS spectra. Antimicrobial assay manifested that some target compounds gave moderate to good antibacterial and antifungal activities. The *N*-1 derived benzotriazole **5e** and *N*-2 derived benzotriazole **6a** exhibited valuable inhibitory efficacy against some tested strains. Especially, derivative **6a** gave superior antifungal efficacies against *C. utilis*, *S. cerevisiae* and *A. flavus* (MIC＝0.01, 0.02, 0.02 μmol/mL, respectively) to Fluconazole. The drug combination of compound **5e** or **6a** with antibacterial Chloromycin, Norfloxacin and antifungal Fluconazole respectively showed stronger antimicrobial efficiency with less dosage and broader antimicrobial spectrum than their separated use alone. The preliminary interaction with calf thymus DNA revealed that compound **6a** could intercalate into DNA to form **6a**-DNA supramolecular complex which might be a factor to exert its powerful bioactivity. Molecular docking study indicated strong binding of compound **6a** with DNA gyrase. The structural parameters such as molecular orbital energy and molecular electrostatic potential of compound **6a** were also investigated, which provided better understanding for its good antimicrobial activity.

**Keywords** chalcone, benzotriazole, antimicrobial, calf thymus DNA, DNA gyrase

## Introduction

Bacterial and fungal infections have been severe threats to global health in the recent decades. This situation has become even aggravated with the emergence of multidrug-resistant strains, intractable pathogenic microorganisms and newly arising pathogens.[1] The discovery and development of novel skeletons with minimal cross-resistance and completely distinct action mechanisms from the well-known class of antimicrobial agents are therefore of great importance to medical community.[2]

Chalcones classically consist of two aromatic rings linked by a three-carbon $\alpha,\beta$-unsaturated system, which exist in numerous medicinal plants and display impressive array of biological properties including antibacterial, antifungal, antiinflammatory, antioxidant, anticancer activities, *etc*.[3] Particularly, their photophysical properties and binding capacity with biomolecules like enzyme or DNA have attracted increasing interest in new drug design and development.[4] Much work has revealed that chalcones as antimicrobial agents possess large potentiality in the treatment of infective diseases. Licochalcone A in Figure 1, a natural chalcone product, has the ability to efficiently inhibit the growth of both *Micrococcus luteus* and *Staphylococcus aureus*. The promising biological profile and easy synthetic accessibility encourage much special interest in investigating chalcone-based compounds as a novel type of potential antimicrobial agents.[5]

Azole compounds as electron-rich nitrogen heterocycles play an extremely important role in medicinal field.[6] Especially in antimicrobial applications, azole compounds have been extensively investigated as antibacterial and antifungal drugs and some of them such as imidazole-based Metronidazole and Sulconazole, triazole-based Fluconazole, tetrazole-based Ceforanide and Cefoperazone, thiazole-based Ceftizoxime and Cefdinir, oxazole-based Oxazolidones as well as pyrazole-based Sulfaphenazole have been employed in

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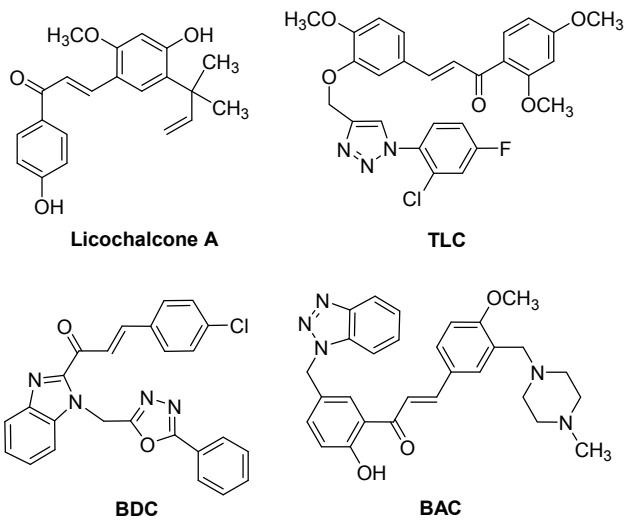


**Figure 1** Structures of some antimicrobial chalcone derivatives.

clinic to treat various types of microbial infections. These exciting observations attract more and more attention on the development of antimicrobial agents containing azole moieties.[7,8] The combination of azole rings with chalcones as antimicrobial agents has been reported actively, which demonstrated that the introduction of nitrogen-containing heterocycles into chalcone backbone is beneficial to exert antimicrobial activities due to the strengthened ability with multiple therapeutic targets.[9] 1,2,3-Triazole-linked chalcone (**TLC**) showed equipotent activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) (MIC＝0.01 μmol/mL) and *Enterococcus faecalis* (MIC ＝ 0.01 μmol/mL) to antibiotic Ciprofloxacin.[10] Benzimidazole-derived chalcone (**BDC**) exhibited comparable inhibitory activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes* to standard drug Chloromycin.[11] All the above observations reveal that the introduction of heterocycles into chalcone skeleton is of great development value as antibacterial and antifungal agents, which has provoked considerable interest to continuously investigate heterocycle-based chalcones as antimicrobial agents.

Benzotriazole is an important benzene-fused 1,2,3-triazole heterocycle with poly-nitrogen electron-rich planar structural feature. This privileged structure enables benzotriazole derivatives to readily bind with various enzymes or receptors in organisms *via* weak interactions, thereby exhibiting broad bioactive spectrum. A large number of benzotriazole derivatives as antimicrobial agents with medicinal potentiality have been actively exploited.[12] Recently, a type of benzotriazole appended chalcone (**BAC**), in which benzotriazole was incorporated on the phenyl group of chalcone fragment *via* a methylene bridge (Figure 1), has been reported to be potent against some Gram-positive bacteria.[13] However, to our best knowledge, the modification on diaryl enone scaffold of chalcones by nitrogen heterocyclic rings especially at $\alpha$-position is rarely reported. Our previous work revealed that the introduction of triazole into $\alpha$-position of chalcones could lead to potent antimicrobial effectiveness that was even superior to that of the reference drugs.[14] These results arouse our renewed interest in exploring antimicrobial compounds with benzotriazole moiety conjugated at $\alpha$-position of chalcones.

In view of above considerations, a series of chalcone-benzotriazole conjugates were designed and presented in Figure 2. These target compounds were expected to have desirable antibacterial and antifungal properties due to the presence of benzotriazolyl substituent with increasing ability to form hydrogen bonding and π-π stacking interactions. Various substitutions (including chloro, fluoro, hydroxyl, methyl, alkoxy and dimethylamino groups) at benzene ring were introduced to investigate their effects on biological activity because of the fact that substituents at aromatic ring could significantly influence the pharmacological properties by regulating lipid-water partition coefficient and binding affinity.[15] Meanwhile, some *N*-2 derived benzotriazoles were also prepared in order to explore the effects of the different positions of benzotriazole ring on antimicrobial activity. The antimicrobial activities for all synthesized compounds were evaluated *in vitro* against eight bacteria and five fungi. It is well-known that the combination therapy can improve treatment efficiency and bioavailability, minimize undesirable side effects, reduce dose-related toxicity and address urgent clinical need caused by multi-drug resistance.[16] Therefore, in this work the combination effects of strongly bioactive compounds with antibacterial Chloromycin, Norfloxacin and antifungal Fluconazole were evaluated *in vitro*, respectively. The interaction of active molecule with calf thymus DNA was also carried out by UV-vis absorption spectroscopy in order to preliminarily investigate the possible antimicrobial action mechanisms.

## Experimental

### Reagents and measurements

Melting points were recorded on X-6 melting point apparatus and uncorrected. TLC analysis was done using precoated silica gel plates. FT-IR spectra were carried out on Bruker RFS100/S spectrophotometer (Bio-Rad, Cambridge, USA) using KBr pellets in the 400－4000 $cm^{-1}$ range. $^1H$ NMR and $^{13}C$ NMR spectra were recorded on Bruker AV 300 spectrometer (Bruker Bio-Spin AG Ltd., Beijing, China) and AVANCE III 600 spectrometer using TMS as an internal standard. The chemical shifts were reported in parts per million, the coupling constants (*J*) were expressed in hertz (Hz) and signals were described as singlet (s), doublet (d), triplet (t) as well as multiplet (m). The following abbreviations were used to designate aryl groups: BT＝benzotriazolyl, Ph＝phenyl. The mass spectra were recorded on LCMS-2010A and the high resolution mass spectra (HRMS)

  

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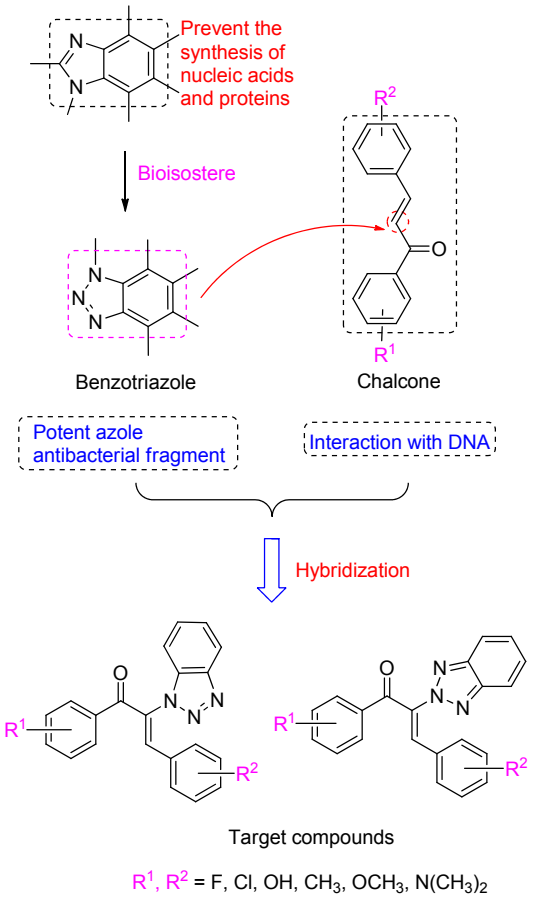


**Figure 2** Design of $\alpha$-benzotriazolyl chalcones.

were recorded on an IonSpec FTICR mass spectrometer with ESI resource. UV spectra were recorded at room temperature on a TU-2450 spectrophotometer (Puxi Analytic Instrument Ltd. of Beijing, China) equipped with 1.0 cm quartz cells. Calf thymus DNA and NR were obtained from Sigma-Aldrich (St. Louis, MO, USA). Tris, HCl were of analytical purity. Sample masses were weighed on a microbalance with a resolution of 0.1 mg. All other chemicals and solvents were commercially available, and used without further purification.

### Synthesis of compounds 2a–2c, 3a–3c and 4a–4b

The intermediates **2a**—**2c**, **3a**—**3c** and **4a**—**4b** were prepared according to the literature procedure.[17]

### Synthesis of (*Z*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(2,4-dichlorophenyl)-3-phenylprop-2-en-1-one (5a)

A mixture of intermediate **3a** (0.608 g, 2.00 mmol) and benzaldehyde (0.212 g, 2.00 mmol) in the presence of glacial acetic acid (0.08 mL, 1.40 mmol) and piperidine (0.08 mL, 1.40 mmol) as catalyst in toluene (50 mL) was stirred under reflux. After the reaction was completed (monitored by TLC, petroleum ether/ethyl acetate, 4/1, *V*/*V*), the solvent was removed under reduced pressure, and the residue was dissolved in trichloromethane and extracted with water. After that, the combined organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography eluting with petroleum ether/ethyl acetate to give the pure compound **5a** (0.616 g) as white crystal. Yield: 79.5%; m.p. 167—169 ℃; IR (KBr) *v*: 3099, 3021 (aromatic C—H, =C—H), 1630 (C=O), 1593 (C=C), 1349, 752 $cm^{-1}$; $^1H$ NMR (600 MHz, $CDCl_3$) $\delta$: 8.14 (d, 1H, $J$=8.3 Hz, 2,4-$Cl_2$Ph-6-*H*), 7.75 (s, 1H, Ph-C*H*), 7.52—7.38 (m, 4H, BT-5,6,7,8-*H*), 7.36 (dd, 1H, $J$=8.2, 1.9 Hz, 2,4-$Cl_2$Ph-5-*H*), 7.32—7.29 (m, 2H, 2,4-$Cl_2$Ph-3-*H*, Ph-4-*H*), 7.16 (t, 2H, $J$=7.9 Hz, Ph-3,5-*H*), 6.80 (d, 2H, $J$=7.5 Hz, Ph-2,6-*H*); $^{13}C$ NMR (151 MHz, $CDCl_3$) $\delta$: 189.44, 145.83, 144.55, 137.31, 135.49, 133.26, 132.15, 132.06, 131.58, 130.93, 130.69, 130.16, 129.96, 129.06, 128.47, 127.52, 124.39, 120.27, 110.14; MS (*m*/*z*): 394.1 $[M+H]^+$; HRMS (ESI) calcd for $C_{21}H_{13}Cl_2N_3O$ $[M + Na]^+$, 416.0333; found 416.0334.

### Synthesis of (*Z*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(2,4-dichlorophenyl)-3-(*p*-tolyl)prop-2-en-1-one (5b)

Compound **5b** (0.158 g) was obtained as white solid according to general procedure described for **5a** starting from 4-methylbenzaldehyde (0.216 g, 1.80 mmol) and compound **3a** (0.534 g, 1.75 mmol). Yield: 22.1%; m.p. 149—150 ℃; IR (KBr) *v*: 3108, 3032 (aromatic C—H, =C—H), 2930 (aliphatic C—H), 1630 (C=O), 1596 (C=C), 1456, 1383, 1349, 747 $cm^{-1}$; $^1H$ NMR (600 MHz, $CDCl_3$) $\delta$: 8.14 (d, 1H, $J$=8.3 Hz, 2,4-$Cl_2$Ph-6-*H*), 7.71 (s, 1H, 4-$CH_3$Ph-C*H*), 7.39 — 7.47 (m, 4H, BT-4,5,6,7-*H*), 7.35 (d, 1H, $J$=8.2 Hz, 2,4-$Cl_2$Ph-5-*H*), 7.31 (d, 1H, $J$=8.2 Hz, 2,4-$Cl_2$Ph-3-*H*), 6.96 (d, 2H, $J$=8.2 Hz, 4-$CH_3$Ph-2,6-*H*), 6.66 (d, 2H, $J$=8.3 Hz, 4-$CH_3$Ph-3,5-*H*), 2.25 (s, 3H, C$H_3$); $^{13}C$ NMR (151 MHz, $CDCl_3$) $\delta$: 189.44, 145.84, 145.07, 143.33, 137.15, 135.65, 133.31, 132.11, 130.93, 130.67, 130.12, 129.89, 129.88, 128.41, 128.14, 127.48, 124.35, 120.24, 110.15, 21.55; HRMS (ESI) calcd for $C_{22}H_{15}Cl_2N_3O$ $[M+H]^+$, 430.0490; found 430.0490.

### Synthesis of (*Z*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-3-(2-chlorophenyl)-1-(2,4-dichlorophenyl)prop-2-en-1-one (5c)

Compound **5c** (0.170 g) was obtained as yellow solid according to general procedure described for **5a** starting from 2-chlorobenzaldehyde (0.210 g, 1.50 mmol) and compound **3a** (0.458 g, 1.50 mmol). Yield: 26.5%; m.p. 132—133 ℃; IR (KBr) *v*: 3133, 3015 (aromatic C—H, =C—H), 1631 (C=O), 1591 (C=C), 1383, 1349, 1283, 741 $cm^{-1}$; $^1H$ NMR (600 MHz, $CDCl_3$) $\delta$: 8.07 (d, 1*H*, $J$=8.3 Hz, 2,4-$Cl_2$Ph-6-*H*), 8.04 (s, 1H, 2-ClPh-C*H*), 7.51 (d, 1H, $J$=8.2 Hz, 2,4-$Cl_2$Ph-5-*H*), 7.46 (s, 1H, 2,4-$Cl_2$Ph-3-*H*), 7.34—7.43 (m, 4H, BT-4,5,6,7-*H*), 7.23 (d, 1H, $J$=8.3 Hz, 2-ClPh-3-*H*), 7.18 (t, 1H, $J$=7.8 Hz, 2-ClPh-4-*H*), 6.83 (t, 1H, $J$=7.4 Hz, 2-ClPh-5-*H*), 6.44 (d, 1H, $J$=8.0 Hz, 2-ClPh-6-*H*); $^{13}C$ NMR (151 MHz, $CDCl_3$) $\delta$: 189.24, 145.62, 139.57, 137.59, 135.42, 135.24, 133.59, 133.18, 132.28, 132.16, 130.18, 138.17, 129.99, 129.96, 129.48, 128.50, 127.58, 127.10, 124.35, 120.24, 110.06; HRMS (ESI) calcd for

  

$C_{21}H_{12}Cl_3N_3O$ [M+Na]$^+$, 449.9944; found 449.9899.

**Synthesis of (*Z*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(2,4-dichlorophenyl)-3-(4-fluorophenyl)prop-2-en-1-one (5d)**

Compound **5d** (0.366 g) was obtained as white solid according to general procedure described for **5a** starting from 4-fluorobenzaldehyde (0.220 g, 1.77 mmol) and compound **3a** (0.510 g, 1.67 mmol). Yield: 53.2%; m.p. 203−205 ℃; IR (KBr) *v*: 3115, 3025 (aromatic C−H, =C−H), 1630 (C=O), 1594 (C=C), 1349, 752 cm$^{-1}$; $^1$H NMR (600 MHz, $CDCl_3$) *δ*: 8.14 (d, 1H, *J*=8.3 Hz, 2,4-$Cl_2$Ph-6-*H*), 7.71 (s, 1H, 4-FPh-C*H*), 7.52−7.39 (m, 4H, BT-4,5,6,7-*H*), 7.35 (d, 1H, *J*=8.2 Hz, 2,4-$Cl_2$Ph-5-*H*), 7.30 (d, 1H, *J*=8.2 Hz, 2,4-$Cl_2$Ph-3-*H*), 6.86 (t, 2H, *J*=12.0 Hz, 4-FPh-2,6-*H*), 6.83−6.78 (m, 2H, 4-FPh-3,5-*H*); $^{13}$C NMR (151 MHz, $CDCl_3$) *δ*: 189.29, 165.39, 163.58, 145.84, 143.12, 137.37, 135.36, 133.02, 132.11, 131.26, 130.17, 129.93, 128.61, 127.54, 127.21, 124.52, 120.34, 116.54, 110.05; HRMS (ESI) calcd for $C_{21}H_{12}Cl_2FN_3O$ [M+Na]$^+$, 434.0239; found 434.0237.

**Synthesis of (*Z*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(2,4-dichlorophenyl)-3-(4-hydroxy-3-methoxyphenyl)-prop-2-en-1-one (5e)**

Compound **5e** (0.173 g) was obtained as yellow solid according to general procedure described for **5a** starting from 4-hydroxy-3-methoxybenzaldehyde (0.152 g, 1.00 mmol) and compound **3a** (0.306 g, 1.00 mmol). Yield: 39.3%; m.p. 178−179 ℃; IR (KBr) *v*: 3424 (OH), 3110, 3046 (aromatic C−H, =C−H), 2934 (aliphatic C−H), 1630 (C=O), 1594 (C=C), 1384, 1349, 758 cm$^{-1}$; $^1$H NMR (600 MHz, $CDCl_3$) *δ*: 8.13 (d, 1H, *J*=8.3 Hz, 2,4-$Cl_2$Ph-6-*H*), 7.66 (s, 1H, 4-OH-3-$OCH_3$Ph-C*H*), 7.49−7.39 (m, 4H, BT-4,5,6,7-*H*), 7.36 (m, 2H, 2,4-$Cl_2$Ph-3,5-*H*), 6.78 (d, 1H, *J*=8.3 Hz, 4-OH-3-$OCH_3$Ph-5-*H*), 6.73 (d, 1H, *J*=8.4 Hz, 4-OH-3-$OCH_3$Ph-6-*H*), 5.61 (s, 1H, 4-OH-3-$OCH_3$Ph-2-*H*), 3.21 (s, 3H, O*CH*$_3$); $^{13}$C NMR (151 MHz, $CDCl_3$) *δ*: 189.34, 150.12, 146.71, 145.82, 145.51, 137.00, 135.85, 133.42, 132.05, 130.11, 129.82, 128.92, 128.55, 128.54, 127.48, 124.52, 123.12, 120.01, 114.78, 110.72, 110.30, 55.30; HRMS (ESI) calcd for $C_{22}H_{15}Cl_2N_3O_3$ [M+H]$^+$, 440.0569; found 440.0569.

**Synthesis of (*Z*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(2,4-difluorophenyl)-3-phenylprop-2-en-1-one (5f)**

Compound **5f** (0.360 g) was obtained as yellow solid according to general procedure described for **5a** starting from benzaldehyde (0.130 g, 1.23 mmol) and compound **3b** (0.335 g, 1.23 mmol). Yield: 81.1%; m.p. 175−176 ℃; IR (KBr) *v*: 3107, 3042 (aromatic C−H, =C−H), 1630 (C=O), 1595 (C=C), 1501, 1453, 1417, 1384, 1349, 751 cm$^{-1}$; $^1$H NMR (600 MHz, $CDCl_3$) *δ*: 8.11 (d, 1H, *J*=7.9 Hz, 2,4-$F_2$Ph-6-*H*), 7.81 (s, 1H, Ph-C*H*), 7.64 (m, 1H, 2,4-$F_2$Ph-3-*H*), 7.42−7.28 (m, 4H, BT-4,5,6,7-*H*), 7.19 (d, 1H, *J*=8.0 Hz, 2,4-$F_2$Ph-5-*H*), 7.16 (t, 2H, *J*=7.7 Hz, Ph-3,5-*H*), 6.97 (t, 1H, *J*=8.0 Hz, Ph-4-*H*), 6.83 (d, 2H, *J*=7.6 Hz, Ph-2,6-*H*); $^{13}$C NMR (151 MHz, $CDCl_3$) *δ*: 187.25, 165.10, 160.45, 145.75, 142.05, 133.04, 132.11, 132.05, 131.66, 131.13, 130.47, 128.99, 128.36, 124.33, 120.25, 112.44, 112.29, 110.14, 104.70; HRMS (ESI) calcd for $C_{21}H_{13}F_2N_3O$ [M+Na]$^+$, 384.0924; found 384.0924.

**Synthesis of (*Z*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(2,4-difluorophenyl)-3-(4-(dimethylamino)phenyl)-prop-2-en-1-one (5g)**

Compound **5g** (0.452 g) was obtained as yellow solid according to general procedure described for **5a** starting from 4-(dimethylamino) benzaldehyde (0.208 g, 1.39 mmol) and compound **3b** (0.383 g, 1.4 mmol). Yield: 74.6%; m.p. 188−189 ℃; IR (KBr) *v*: 3110, 3011 (aromatic C−H, =C−H), 2945 (aliphatic C−H), 1630 (C=O), 1594 (C=C), 1383, 1349, 761 cm$^{-1}$; $^1$H NMR (600 MHz, $CDCl_3$) *δ*: 8.13 (d, 1H, *J*=8.3 Hz, 2,4-$F_2$Ph-6-*H*), 7.74 (s, 1H, 4-N($CH_3$)$_2$Ph-C*H*), 7.58−7.28 (m, 4H, BT-4,5,6,7-*H*), 6.94 (m, 1H, 2,4-$F_2$Ph-5-*H*), 6.84 (m, 1H, 2,4-$F_2$Ph-3-*H*), 6.56 (d, 2H, *J*=9.0 Hz, 4-N($CH_3$)$_2$Ph-2,6-*H*), 6.37 (d, 2H, *J* = 9.1 Hz, 4-N($CH_3$)$_2$Ph-3,5-*H*), 2.94 (s, 6H, N(C*H*$_3$)$_2$); $^{13}$C NMR (151 MHz, $CDCl_3$) *δ*: 186.93, 164.45, 160.06, 152.76, 145.87, 145.11, 133.63, 133.52, 131.45, 128.10, 126.58, 124.11, 120.06, 118.26, 112.00, 111.88, 111.69, 110.26, 104.52, 39.83; HRMS (ESI) calcd for $C_{23}H_{18}F_2N_4O$ [M+Na]$^+$, 427.1346; found 427.1350.

**Synthesis of (*Z*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(2,4-difluorophenyl)-3-(4-hydroxy-3-methoxyphenyl)-prop-2-en-1-one (5h)**

Compound **5h** (0.220 g) was obtained as yellow solid according to general procedure described for **5a** starting from 4-hydroxy-3-methoxybenzaldehyde (0.213 g, 1.40 mmol) and compound **3b** (0.383 g, 1.40 mmol). Yield: 38.8%; m.p. 107−109 ℃; IR (KBr) *v*: 3426 (OH), 3108, 3031 (aromatic C−H, =C−H), 2940 (aliphatic C−H), 1630 (C=O), 1594 (C=C), 1517, 1349, 749 cm$^{-1}$; $^1$H NMR (600 MHz, $CDCl_3$) *δ*: 8.11 (d, 1H, *J*=8.3 Hz, 2,4-$F_2$Ph-6-*H*), 7.75 (s, 1H, 4-OH-3-$OCH_3$-Ph-C*H*), 7.64−7.60, 7.46−7.27 (m, 4H, BT-4,5,6,7-*H*), 6.97 (t, 1H, *J*=8.2 Hz, 2,4-$F_2$Ph-5-*H*), 6.84, 6.84−6.72 (m, 3H, 4-OH-3-$OCH_3$-Ph-2,5,6-*H*), 5.66 (s, 1H, 2,4-$F_2$Ph-3-*H*), 3.21 (s, 3H, O*CH*$_3$); $^{13}$C NMR (151 MHz, $CDCl_3$) *δ*: 182.43, 160.13, 155.56, 145.06, 141.93, 140.99, 138.83, 128.56, 123.70, 123.37, 119.70, 118.47, 115.24, 115.03, 113.79, 110.01, 109.81, 107.48, 105.99, 105.57, 99.91, 50.41; HRMS (ESI) calcd for $C_{22}H_{15}F_2N_3O_3$ [M+H]$^+$, 408.1160; found 408.1158.

**Synthesis of (*Z*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(3,4-dimethylphenyl)-3-phenylprop-2-en-1-one (5i)**

Compound **5i** (0.095 g) was obtained as yellow solid according to general procedure described for **5a** starting from benzaldehyde (0.106 g, 1.00 mmol) and compound **3c** (0.265 g, 1.00 mmol). Yield: 27.0%; m.p. 108−110 ℃; IR (KBr) *v*: 3122, 3011 (aromatic C−H, =C−H), 2958 (aliphatic C−H), 1631 (C=O), 1595 (C=C), 1383, 1349 cm$^{-1}$; $^1$H NMR (600 MHz, $CDCl_3$) *δ*:

  

8.13 (d, 1H, $J$=8.2 Hz, 3,4-$(CH_3)_2$Ph-6-*H*), 7.71 (s, 1H, Ph-C*H*), 7.47−7.27 (m, 4H, BT-4,5,6,7-*H*), 7.22 (d, 1H, $J$=8.2 Hz, 3,4-$(CH_3)_2$Ph-5-*H*), 7.14 (t, 2H, $J$=7.8 Hz, Ph-3,5-*H*), 7.12 (s, 1H, 3,4-$(CH_3)_2$Ph-2-*H*), 7.04 (d, 1H, $J$=7.7 Hz, Ph-4-*H*), 6.80 (d, 2H, $J$=7.8 Hz, Ph-2,6-*H*), 2.47 (s, 3H, 3,4-$(CH_3)_2$Ph-4-C$H_3$), 2.37 (s, 3H, 3,4-$(CH_3)_2$Ph-3-C$H_3$); $^{13}$C NMR (151 MHz, $CDCl_3$) $\delta$: 193.16, 145.84, 142.99, 141.39, 137.43, 134.08, 133.37, 132.76, 132.23, 131.39, 131.31, 130.32, 128.94, 128.4, 128.25, 126.12, 124.18, 120.22, 109.94, 21.38, 19.93; HRMS (ESI) calcd for $C_{23}H_{19}N_3O$ $[M+H]^+$, 354.1606; found 354.1603.

**Synthesis of (*Z*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(3,4-dimethylphenyl)-3-(4-fluorophenyl)prop-2-en-1-one (5j)**

Compound **5j** (0.271 g) was obtained as white solid according to general procedure described for **5a** starting from 4-fluorobenzaldehyde (0.162 g, 1.30 mmol) and compound **3c** (0.344 g, 1.30 mmol). Yield: 56.2%; m.p. 194−195 ℃; IR (KBr) $v$: 3114, 3051 (aromatic C−H, =C−H), 2961 (aliphatic C−H), 1631 (C=O), 1595 (C=C), 1383, 1349 cm$^{-1}$; $^1$H NMR (600 MHz, $CDCl_3$) $\delta$: 8.14 (d, 1H, $J$=8.2 Hz, 3,4-$(CH_3)_2$Ph-6-*H*), 7.67 (s, 1H, 4-FPh-C*H*), 7.46−7.39, 7.04 (m, 4H, BT-4,5,6,7-*H*), 7.23 (d, 1H, $J$=8.1 Hz, 3,4-$(CH_3)_2$Ph-5-*H*), 7.12 (s, 1H, 3,4-$(CH_3)_2$Ph-2-*H*), 6.84 (t, 2H, $J$=8.5 Hz, 4-FPh-2,6-*H*), 6.81−6.77 (m, 2H, 4-FPh-3,5-*H*), 2.47 (s, 3H, 3,4-$(CH_3)_2$Ph-4-C$H_3$), 2.36 (s, 3H, 3,4-$(CH_3)_2$Ph-3-C$H_3$); $^{13}$C NMR (151 MHz, $CDCl_3$) $\delta$: 192.97, 164.22, 145.86, 141.62, 141.45, 137.45, 133.93, 133.27, 132.60, 132.45, 132.25, 128.38, 127.57, 126.11, 124.31, 120.32, 116.36, 116.21, 109.83, 21.37, 19.91; HRMS (ESI) calcd for $C_{23}H_{18}FN_3O$ $[M+H]^+$, 394.1332; found 394.1331.

**Synthesis of (*Z*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(3,4-dimethylphenyl)-3-(4-hydroxy-3-methoxyphenyl)-prop-2-en-1-one (5k)**

Compound **5k** (0.381 g) was obtained as yellow solid according to general procedure described for **5a** starting from 4-hydroxy-3-methoxybenzaldehyde (0.198 g, 1.30 mmol) and compound **3c** (0.345 g, 1.30 mmol). Yield: 73.5%; m.p. 176−178 ℃; IR (KBr) $v$: 3420 (OH), 3097, 3021 (aromatic C−H, =C−H), 2967 (aliphatic C−H), 1633 (C=O), 1595 (C=C), 1349 cm$^{-1}$; $^1$H NMR (600 MHz, $CDCl_3$) $\delta$: 8.13 (d, 1H, $J$=8.3 Hz, 3,4-$(CH_3)_2$Ph-6-*H*), 7.65 (s, 1H, 4-OH-3-$OCH_3$-Ph-C*H*), 7.47−7.27 (m, 4H, BT-4,5,6,7-*H*), 7.12 (s, 1H, 3,4-$(CH_3)_2$Ph-2-*H*), 7.05 (d, 1H, $J$=7.7 Hz, 3,4-$(CH_3)_2$-Ph-5-*H*), 6.75 (d, 1H, $J$=8.3 Hz, 4-OH-3-$OCH_3$Ph-5-*H*), 6.68 (d, 1H, $J$=8.3 Hz, 4-OH-3-$OCH_3$Ph-6-*H*), 5.64 (s, 1H, 4-OH-3-$OCH_3$Ph-2-*H*), 3.21 (s, 3H, O$CH_3$), 2.46 (s, 3H, 3,4-$(CH_3)_2$Ph-4-C$H_3$), 2.37 (s, 3H, 3,4-$(CH_3)_2$Ph-3-C$H_3$); $^{13}$C NMR (151 MHz, $CDCl_3$) $\delta$: 193.33, 149.48, 146.66, 145.81, 144.17, 140.95, 136.98, 134.53, 133.54, 132.07, 130.19, 128.32, 128.15, 127.73, 126.11, 124.32, 123.38, 119.97, 114.65, 110.60, 110.17, 55.28, 21.35, 19.82; HRMS (ESI) calcd for $C_{24}H_{21}N_3O_3$ $[M+H]^+$, 422.1481; found 422.1486.

**Sythesis of (*Z*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-3-(4-(dimethylamino)phenyl)-1-(3,4-dimethylphenyl)-prop-2-en-1-one (5l)**

Compound **5l** (0.315 g) was obtained as yellow crystal according to general procedure described for **5a** starting from 4-(dimethylamino)benzaldehyde (0.164 g, 1.10 mmol) and compound **3c** (0.291 g, 1.10 mmol). Yield: 72.3%; m.p. 195−196 ℃; IR (KBr) $v$: 3114, 3041 (aromatic C−H, =C−H), 2970 (aliphatic C−H), 1631 (C=O), 1593 (C=C), 1383, 1349 cm$^{-1}$; $^1$H NMR (600 MHz, $CDCl_3$) $\delta$: 8.14 (d, 1H, $J$=8.0 Hz, 3,4-$(CH_3)_2$Ph-6-*H*), 7.64 (s, 1H, 4-N$(CH_3)_2$Ph-C*H*), 7.44−7.3 (m, 4H, BT-4,5,6,7-*H*), 7.09 [s, 1H, 3,4-$(CH_3)_2$Ph-2-*H*], 7.03 [d, 1H, $J$=7.6 Hz, 3,4-$(CH_3)_2$Ph-5-*H*], 6.53 (d, 2H, $J$=8.6 Hz, 4-N$(CH_3)_2$Ph-2,6-*H*), 6.36 (d, 2H, $J$=8.7 Hz, 4-N$(CH_3)_2$Ph-3,5-*H*), 2.92 (s, 6H, N$(CH_3)_2$), 2.44 (s, 3H, 3,4-$(CH_3)_2$Ph-4-C$H_3$), 2.36 (s, 3H, 3,4-$(CH_3)_2$Ph-3-C$H_3$); $^{13}$C NMR (151 MHz, $CDCl_3$) $\delta$: 193.31, 152.33, 145.94, 145.20, 140.25, 136.53, 135.34, 133.71, 133.21, 132.91, 131.83, 127.94, 127.72, 125.99, 123.94, 120.07, 118.73, 111.83, 110.15, 39.92, 21.33, 19.74; HRMS (ESI) calcd for $C_{25}H_{24}N_4O$ $[M+H]^+$, 419.1848; found 419.1850.

**Synthesis of (*Z*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(4-fluoro-2-(piperidin-1-yl)phenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (5m)**

Compound **5m** (0.170 g) was obtained as yellow solid from procedure for the preparation of compound **5h**. Yield: 25.7%; m.p. 204−206 ℃; IR (KBr) $v$: 3427 (OH), 3132, 3056 (aromatic C−H, =C−H), 2942 (aliphatic C−H), 2862 ($CH_2$), 1630 (C=O), 1593 (C=C), 1513, 1349, 749 cm$^{-1}$; $^1$H NMR (600 MHz, $CDCl_3$) $\delta$: 8.09 (d, $J$=8.2 Hz, 1H, 4-F-2-$C_5H_{10}$N-Ph-6-*H*), 7.71 (s, 1H, 4-OH-3-$OCH_3$Ph-C*H*), 7.44−7.35, 7.23 (m, 4H, BT-4,5,6,7-*H*), 6.77 (d, 1H, $J$=8.3 Hz, 4-F-2-$C_5H_{10}$N-Ph-5-*H*), 6.73−6.66 (m, 3H, 4-OH-3-$OCH_3$Ph-2,5,6-*H*), 6.13 (s, 1H, 4-OH-3-$OCH_3$Ph-3-O*H*), 5.60 (s, 1H, 4-F-2-$C_5H_{10}$N-Ph-3-*H*), 3.18 (s, 3H, O$CH_3$), 3.01 (t, 4H, $C_5H_{10}$N-2,6-2*H*), 1.66 (m, 4H, $C_5H_{10}$N-3,5-2*H*), 1.51 (m, 2H, $C_5H_{10}$N-4-*H*); $^{13}$C NMR (151 MHz, $CDCl_3$) $\delta$: 192.59, 165.58, 154.32, 149.39, 146.78, 145.65, 143.23, 133.67, 132.06, 131.99, 128.94, 128.03, 127.63, 124.22, 123.66, 119.86, 114.75, 110.61, 110.46, 108.39, 105.91, 55.27, 53.78, 25.87, 23.99; HRMS (ESI) calcd for $C_{27}H_{25}FN_4O_3$ $[M+H]^+$, 495.1808; found 495.1811.

**Synthesis of (*Z*)-2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-3-(3-ethoxy-4-hydroxyphenyl)-1-(4-fluoro-2-(piperidin-1-yl)phenyl)prop-2-en-1-one (5n)**

Compound **5n** (0.280 g) was obtained as yellow solid according to general procedure described for **5a** starting from 3-ethoxy-4-hydroxybenzaldehyde (0.230 g, 1.39 mmol) and compound **3b** (0.374 g, 1.37 mmol). Yield: 42.0%; m.p. 197−198 ℃; IR (KBr) $v$: 3426 (OH), 3115, 3031 (aromatic C−H, =C−H), 2925, 2850 (aliphatic C−H), 1630 (C=O), 1594 (C=C), 1455, 1383, 1349, 738 cm$^{-1}$; $^1$H NMR (600 MHz, $CDCl_3$) $\delta$: 8.10 (d, 1H, $J$=8.2 Hz, 4-F-2-$C_5H_{10}$N-Ph-6-

  

*H*), 7.71 (s, 1H, 4-OH-3-$OCH_2CH_3$Ph-C*H*), 7.49－7.32, 7.24 (m, 4H, BT-4,5,6,7-*H*), 6.78 (d, *J*＝8.3 Hz, 1H, 4-F-2-$C_5H_{10}$N-Ph-5-*H*), 6.75－6.67 (m, 3H, 4-OH-3-O-$CH_2CH_3$Ph-2,5,6-*H*), 6.02 (s, 1H, 4-OH-3-$OCH_2CH_3$Ph-4-O*H*), 5.58 (s, 1H, 4-F-2-$C_5H_{10}$N-Ph-3-*H*), 3.31 (m, 2H, $OCH_2CH_3$), 3.03 (t, *J*＝4.3 Hz, 4H, $C_5H_{10}$N-2,6-2*H*), 1.67 (m, 4H, $C_5H_{10}$N-3,5-2*H*), 1.51 (m, 2H, $C_5H_{10}$N-4-*H*), 1.08 (t, 3H, *J*＝7.0 Hz, $OCH_2CH_3$); $^{13}$C NMR (151 MHz, $CDCl_3$) *δ*: 192.53, 165.02, 154.18, 149.43, 145.91, 145.65, 143.37, 133.63, 132.03, 131.97, 128.78, 127.99, 127.52, 124.15, 123.58, 119.82, 114.62, 111.27, 110.39, 108.31, 105.97, 64.04, 53.75, 25.81, 23.93, 14.27; HRMS (ESI) calcd for $C_{28}H_{27}FN_4O_3$ $[M+H]^+$, 487.2145; found 487.2147.

**Synthesis of (*Z*)-2-(2*H*-benzo[*d*][1,2,3]triazol-2-yl)-1-(2,4-difluorophenyl)-3-phenylprop-2-en-1-one (6a)**

Compound **6a** (0.143 g) was obtained as white solid according to general procedure described for **5a** starting from benzaldehyde (0.212 g, 2.00 mmol) and compound **4a** (0.546 g, 2.00 mmol). Yield: 19.9%; m.p. 118－120 ℃; IR (KBr) *v*: 3132, 3045 (aromatic C－H, ＝C－H), 1630 (C＝O), 1600 (C＝C), 1383, 1348, 741 $cm^{-1}$; $^1$H NMR (600 MHz, $CDCl_3$) *δ*: 8.11 (d, 1H, 8.5 Hz, 2,4-$F_2$Ph-6-*H*), 8.05 (s, 1H, Ph-C*H*), 7.84 (d, *J*＝6.6, 2H, BT-4,7-*H*), 7.43－7.36 (m, 4H, BT-5,6-*H*, Ph-2,6-*H*), 7.29 (m, 3H, Ph-3,4,5-*H*), 6.94 (m, 1H, 2,4-$F_2$Ph-5-*H*), 6.71 (m, 1H, 2,4-$F_2$Ph-3-*H*); $^{13}$C NMR (151 MHz, $CDCl_3$) *δ*: 185.11, 166.63, 163.13, 144.74, 137.45, 133.59, 132.19, 130.67, 129.40, 129.34, 128.81, 127.57, 125.61, 118.29, 112.48, 105.18; HRMS (ESI) calcd for $C_{21}H_{13}F_2N_3O$ $[M+H]^+$, 362.1105; found 362.1105.

**Synthesis of (*Z*)-2-(2*H*-benzo[*d*][1,2,3]triazol-2-yl)-1-(3,4-dimethylphenyl)-3-(4-fluorophenyl)prop-2-en-1-one (6b)**

Compound **6b** (0.190 g) was obtained as yellow solid according to general procedure described for **5a** starting from 4-fluorobenzaldehyde (0.248 g, 2.00 mmol) and compound **4b** (0.530 g, 2.00 mmol). Yield: 25.6%; m.p. 145－146 ℃; IR (KBr) *v*: 3110, 3041 (aromatic C－H, ＝C－H), 2965 (aliphatic C－H), 1631(C＝O), 1595 (C＝C), 1411, 1383, 1349, 778 $cm^{-1}$; $^1$H NMR (600 MHz, $CDCl_3$) *δ*: 7.94 (dd, 2H, *J*＝6.6, 3.1 Hz, BT-4,7-*H*), 7.58 (s, 1H, 4-FPh-C*H*), 7.47 (d, 1H, *J*＝7.8 Hz, 3,4-$(CH_3)_2$Ph-6-*H*), 7.45 (dd, 2H, *J*＝6.6, 3.1 Hz, BT-5,6-*H*), 7.12 (s, 1H, 3,4-$(CH_3)_2$Ph-2*H*), 7.04 (d, 1H, *J*＝7.7 Hz, 3,4-$(CH_3)_2$Ph-5-*H*), 6.84 (t, 2H, *J*＝8.6 Hz, 4-FPh-2,6-*H*), 6.67 (dd, 2H, *J*＝8.8, 5.4 Hz, 4-FPh-3,5-*H*), 2.48 (s, 3H, 3,4-$(CH_3)_2$-Ph-3-C$H_3$), 2.36 (s, 3H, 3,4-$(CH_3)_2$-Ph-4-C$H_3$); $^{13}$C NMR (151 MHz, $CDCl_3$) *δ*: 192.31, 165.19, 163.50, 145.13, 141.36, 141.03, 137.63, 133.91, 132.67, 132.17, 128.48, 127.29, 126.09, 118.72, 116.26, 116.12, 21.37, 19.88; HRMS (ESI) calcd for $C_{23}H_{18}FN_3O$ $[M+H]^+$, 394.1332; found 394.1329.

**Synthesis of (*Z*)-2-(2*H*-benzo[*d*][1,2,3]triazol-2-yl)-1-(4-fluoro-2-(piperidin-1-yl)phenyl)-3-phenyl-prop-2-en-1-one (6c)**

Compound **6c** (0.193 g) was obtained as white solid from procedure for the preparation of compound **6a**. Yield: 22.6%; m.p. 150－152 ℃; IR (KBr) *v*: 3111, 3051 (aromatic C－H, ＝C－H), 2855 (aliphatic C－H), 1630 (C＝O), 1596 (C＝C), 1455, 1383, 1349, 738 $cm^{-1}$; $^1$H NMR (600 MHz, $CDCl_3$) *δ*: 7.90 (dd, *J*＝6.6, 3.0 Hz, 2H, BT-4,7-*H*), 7.69 (s, 1H, Ph-C*H*), 7.48 (d, *J*＝8.2, 1H, 4-F-2-$C_5H_{10}$N-Ph-6-*H*), 7.42 (dd, *J*＝6.6, 3.0 Hz, 1H, BT-5,6-*H*), 7.29 (t, *J*＝7.4 Hz, 1H, Ph-4-*H*), 7.15 (t, *J*＝7.8 Hz, 2H, Ph-2,6-*H*), 6.74－6.65 (m, 4H, 4-F-2-$C_5H_{10}$N-Ph-3,5-*H*, Ph-3,5-*H*), 3.02 (t, *J*＝6 Hz, 4H, $C_5H_{10}$N-2,6-2*H*), 1.69 (m, 4H, $C_5H_{10}$N-3,5-2*H*), 1.52 (m, 2H, $C_5H_{10}$N-4-*H*); $^{13}$C NMR (151 MHz, $CDCl_3$) *δ*: 191.67, 165.23, 144.88, 141.76, 136.45, 132.58, 132.51, 131.38, 131.22, 130.35, 128.83, 126.98, 118.69, 118.18, 108.22, 105.56, 53.71, 25.76, 23.97; HRMS (ESI) calcd for $C_{26}H_{23}FN_4O$ $[M+H]^+$, 427.1934; found 427.1937.

**Biological assays**

The *in vitro* minimal inhibitory concentrations (MICs) of the target compounds were determined using the two-fold serial dilution technique in 96-well microtest plates, according to the National Committee for Clinical Laboratory Standards (NCCLS).[18] The tested microorganism strains were provided by the School of Pharmaceutical Sciences, Southwest University and the College of Pharmacy, Third Military Medical University, China. Chloromycin, Norfloxacin and Fluconazole were used as standard drugs.

The prepared compounds **5a**－**5n** and **6a**－**6c** were evaluated for their antibacterial activities against Gram-positive bacteria (Methicillin-resistant *Staphylococcus aureus* N315 (MRSA), *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC6633 and *Micrococcus luteus* ATCC4698), and Gram-negative bacteria (*Bacillus proteus* ATCC13315, *Escherichia coli* DH52, *Pseudomonas aeruginosa* ATCC27853 and *Bacillus typhi*). The bacterial suspension was adjusted with sterile saline to a concentration of $1\times10^{-5}$ CFU. The tested compounds were dissolved in DMSO to prepare the stock solutions. The tested compounds and reference drugs were prepared in Mueller–Hinton broth (Guangdong Huaikai Microbial Sci & Tech Co., Ltd, Guangzhou, Guangdong, China) by twofold serial dilution to obtain the various concentrations. These dilutions were inoculated and incubated at 37 ℃ for 24 h. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment. The minimum inhibitory concentration values (MICs) (in μg/mL) for **5a**－**5n** and **6a**－**6c** were summarized in Table1.

The newly synthesized compounds **5a**－**5n** and **6a**－**6c** were evaluated for their antifungal activities against *Candida albicans* ATCC10231, *Candida mycoderma* ATCC9888, *Candida utilis* ATCC9950, *Saccharomyces cerevisiae* ATCC9763 and *Aspergillus flavus* ATCC204304. A spore suspension in sterile distilled

  

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water was prepared from a culture of the fungi growing on Sabouraud agar (SA) media, aged 1 d. The final spore concentration was $1\times10^3-5\times10^3$ spore/mL. From the stock solutions of the tested compounds and reference antifungal drug Fluconazole, dilutions in sterile RPMI 1640 medium (Neuronbc Laboraton Technology Co., Ltd., Beijing, China) were made, resulting in eleven desired concentrations (0.5 to 512 μg/mL) of each tested compound. These dilutions were inoculated and incubated at 35 ℃ for 24 h.

## Results and Discussion

### Chemistry

The target *α*-benzotriazolyl chalcones were synthesized from commercially available substituted benzenes, chloroacetyl chloride, benzotriazole and aromatic aldehydes. The synthetic route was outlined in Scheme 1. Intermediates **2a**—**2c** were prepared by the Friedel-Crafts acylation of substituted benzenes **1a**—**1c** respectively with chloroacetyl chloride in dichloromethane with excellent yields of 90%—98%, which were further reacted with 1*H*-benzotriazole under basic conditions to give corresponding *N*-1 alkylated benzotriazole derivatives **3a**—**3c** (34%—67%) and *N*-2 substituted benzotriazole derivatives **4a**—**4b** (8%—15%) through nucleophilic substitution. During the *N*-alkylations of benzotriazole with alkyl halides, the lower reaction temperature favored the *N*-1 orientation while the higher reaction temperature favored the *N*-2 orientation. Interestingly, *N*-2 substituted dichlorophenyl benzotriazole could not be obtained by the same procedure. The aldol condensation of intermediates **3a**—**3c** and **4a**—**4b** respectively with equimolar aromatic aldehydes in toluene by the catalysis of piperidine and glacial acetic acid afforded target compounds **5a**—**5l** and **6a**—**6b** in 20%—80% yields.

The absolute configuration of compound **5a** was confirmed by single crystal X-ray diffraction analysis (Figure 3). Accidentally, piperidyl substituted chalcones **5m** and **6c** were obtained as byproducts in the procedure for preparing compounds **5h** and **6a**, respectively. In contrast, compound **5n** was obtained as the main product in aldol condensation reaction.

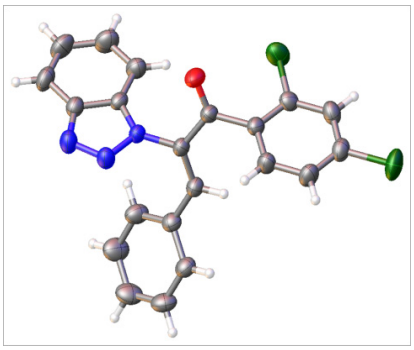


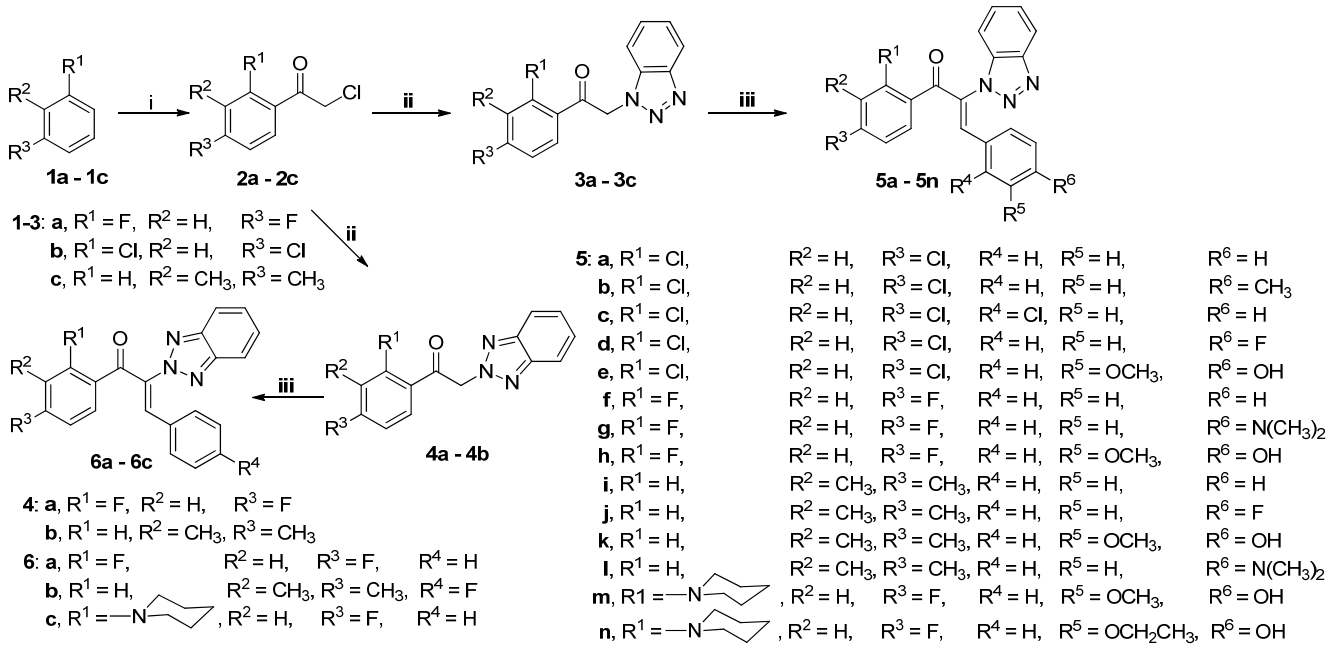
**Figure 3** X-ray structure of benzotriazolyl chalcone **5a**.

### Spectral analysis

All the newly synthesized compounds were characterized by IR, $^1$H NMR, $^{13}$C NMR and HRMS spectra. The spectral analyses were in accordance with the assigned structures, and listed in the experimental section.

In IR spectra, the prominent characteristic C=O bands of all the synthesized benzotriazole compounds

**Scheme 1**




**Reagents and conditions**: (i) $ClCOCH_2Cl$, $AlCl_3$, $CH_2Cl_2$, r.t.; (ii) benzotriazole, $K_2CO_3$, $CH_3CN$, r.t.—65 ℃; (iii) aryl aldehydes, glacial acetic acid and piperidine, toluene, reflux, 3–4 h.

appeared in the region between 1634 and 1600 $cm^{-1}$. The sharp and strong peaks in the region between 1600 and 1591 $cm^{-1}$ were attributed to the characteristic C=C bands of all compounds. In addition, compounds **5e**, **5h**, **5k**, **5m** and **5n** gave broad absorption in 3427—3420 $cm^{-1}$ which indicated the presence of OH group in benzene ring. Peaks at 2970—2850 $cm^{-1}$ in compounds **5b**, **5e**, **5g**—**5n** and **6b**—**6c** were ascribed to the stretching vibrations of aliphatic C—H bond. All the other absorption bands were also observed at the expected regions.

The $^1$H NMR spectra revealed that singlet at the region of $\delta$ 7.58—8.05 was assigned to the Ar—C*H*=C in all title compounds. It was observed that the peaks of protons 4,7-*H* and 5,6-*H* in benzotriazole ring for *N*-2 derived benzotriazoles **6a**—**6c** appeared at $\delta$ 7.94—7.84 and 7.45 — 7.38 respectively. Protons of piperidyl groups in **5m**—**5n** and **6c** displayed reasonable chemical shift values at aliphatic region. Additionally, all aromatic and aliphatic protons appeared at the appropriate chemical shifts and integral.

The $^{13}$C NMR spectral analyses were in accordance with the assigned structures. In compounds **5f**—5**h** and **6a**, the split signals observed at $\delta$ 165.97—159.28 and 161.31—154.68 were assigned to the *C*-2 and *C*-4 positions of 2,4-difluorophenyl group respectively due to the existence of F atom with strong electronegativity. The signals at $\delta$ 193.33—185.11 in all the title compounds were ascribed to the carbonyl carbon. The O-*C*$H_3$ and N-*C*$H_3$ moieties in compounds **5e**, **5g**—**5h** and **5k**—**5m** gave upfield chemical shift at $\delta$ 55.30–39.83 in contrast to methyl attached to aromatic ring in compounds **5b**, **5i**—**5j** and **6b** ($\delta$ 21.55—19.74), which were attributed to the strong electron withdrawing characters of *O* and *N* atom. In addition, the carbon signal of piperidyl group in compounds **5m**—**5n** and **6c** appeared in the appropriate aliphatic regions. All the other carbons gave $^{13}$C peaks at the expected regions.

### Analysis of clog *P* values

Hydrophobic/lipophilic property has been prevalently considered as an important factor in drug design and development due to its effect on the interactions between molecule and membrane. The calculated liposome/water partition coefficients (clog *P*) for all the newly prepared compounds were shown in Table 1. The results revealed that all the target compounds had clog *P* values between 3.57 and 6.43, suggesting that the newly

**Table 1** clog *P* values and antimicrobial data as MIC (μmol/mL) for compounds **5**—**6**[a,b,c,d]

| Compd | clog *P* | Gram-positive bacteria | | | | Gram-negative bacteria | | | | Fungi | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S. A | MRSA | B. S | M. L | B. P | E. C | P. A | B. T | C. A | C. M | C. U | S. C | A. F |
| **5a** | 5.72 | 0.04 | 0.16 | 0.16 | 0.02 | 0.65 | 0.04 | 1.30 | 0.65 | 0.04 | 0.04 | 1.3 | 0.65 | 0.65 |
| **5b** | 6.22 | 1.26 | 0.08 | 0.08 | 0.16 | 1.26 | 1.26 | 0.31 | 0.31 | 1.26 | 0.31 | 1.26 | 1.26 | 1.26 |
| **5c** | 6.43 | 1.20 | 0.07 | 0.60 | 0.001 | 1.20 | 0.30 | 0.15 | 0.15 | 1.20 | 1.20 | 1.20 | 0.08 | 1.20 |
| **5d** | 5.86 | 1.25 | 0.16 | 0.31 | 1.25 | 1.25 | 1.25 | 0.31 | 0.08 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 |
| **5e** | 4.9 | 0.009 | 1.17 | 0.29 | 0.04 | 1.17 | 0.07 | 1.17 | 0.29 | 0.009 | 0.04 | 0.009 | 0.15 | 0.07 |
| **5f** | 4.39 | 0.36 | 0.36 | 0.36 | 0.09 | 0.36 | 0.71 | 0.09 | 0.71 | 0.36 | 0.71 | 0.04 | 0.71 | 0.09 |
| **5g** | 4.55 | 0.08 | 1.27 | 0.08 | 0.32 | 1.27 | 0.63 | 1.27 | 1.27 | 0.08 | 1.27 | 1.27 | 1.27 | 1.27 |
| **5h** | 3.57 | 0.08 | 1.26 | 1.26 | 0.02 | 0.16 | 0.16 | 0.31 | 0.31 | 0.08 | 0.31 | 0.04 | 0.16 | 0.31 |
| **5i** | 5.4 | 0.09 | 1.45 | 0.36 | 0.36 | 0.09 | 0.02 | 0.36 | 0.36 | 0.09 | 0.18 | 0.01 | 1.45 | 0.73 |
| **5j** | 5.54 | 0.69 | 1.38 | 1.38 | 1.38 | 1.38 | 0.35 | 1.38 | 1.38 | 0.69 | 1.38 | 1.38 | 1.38 | 0.35 |
| **5k** | 4.58 | 1.28 | 0.04 | 1.28 | 0.32 | 0.08 | 0.16 | 1.28 | 0.64 | 1.28 | 0.08 | 1.28 | 0.64 | 0.08 |
| **5l** | 5.56 | 0.65 | 0.65 | 1.29 | 0.65 | 1.29 | 0.65 | 1.29 | 0.65 | 0.65 | 1.29 | 1.29 | 1.29 | 0.65 |
| **5m** | 5.01 | 0.14 | 0.54 | 0.14 | 0.27 | 0.14 | 0.14 | 1.08 | 0.27 | 0.14 | 0.07 | 0.14 | 0.07 | 0.03 |
| **5n** | 5.54 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 |
| **6a** | 4.72 | 0.04 | 0.71 | 1.42 | 0.04 | 0.02 | 0.02 | 0.04 | 0.36 | 0.04 | 0.02 | 0.01 | 0.02 | 0.02 |
| **6b** | 5.88 | 0.17 | 0.69 | 0.35 | 0.09 | 0.69 | 1.38 | 0.17 | 0.17 | 0.17 | 0.04 | 0.02 | 0.02 | 0.04 |
| **6c** | 6.16 | 1.20 | 1.20 | 0.30 | 1.20 | 1.20 | 1.20 | 0.30 | 1.20 | 1.20 | 0.005 | 0.60 | 1.20 | 0.30 |
| **A** | –1.09 | 0.02 | 0.05 | 0.10 | 0.02 | 0.10 | 0.05 | 0.05 | 0.10 | — | — | — | — | — |
| **B** | 0.58 | 0.03 | 0.003 | 0.006 | 0.003 | 0.01 | 0.003 | 0.003 | 0.003 | — | — | — | — | — |
| **C** | — | — | — | — | — | — | — | — | — | 0.003 | 0.01 | 0.03 | 0.05 | 0.84 |

[a] Minimum inhibitory concentration (MIC) was determined by micro broth dilution method for microdilution plates. [b] S. A, *Staphylococcus aureus* ATCC25923; MRSA, Methicillin-resistant *Staphylococcus aureus* N315; B. S, *Bacillus subtilis* ATCC6633; M. L, *Micrococcus luteus* ATCC4698; B. P, *Bacillus proteus* ATCC13315; E. C, *Escherichia coli* DH52; P. A, *Pseudomonas aeruginosa* ATCC27853; B. T, *Bacillus typhi*; C. A, *Candida albicans* ATCC10231; C. M, *Candida mycoderma* ATCC9888; C. U, *Candida utilis* ATCC9950; S. C, *Saccharomyces cerevisiae* ATCC9763; A. flavus, *Aspergillus flavus* ATCC204304. [c] **A**=Chloromycin, **B**=Norfloxacin, **C**=Fluconazole. [d] clog *P* values were calculated by ChemDraw Ultra 12.

synthesized compounds possessed suitable lipophilicity, which were favorable for them to permeate through biological membrane and to be delivered to binding sites in organisms.[19]

**Biological activity**

The *in vitro* antimicrobial screening for all the synthesized compounds was evaluated against four Gram-positive bacteria, four Gram-negative bacteria and five fungi using two fold serial dilution technique recommended by National Committee for Clinical Laboratory Standards (NCCLS) with the positive control of clinical antimicrobial drugs Chloromycin, Norfloxacin and Fluconazole. The drug combination studies were screened by checkerboard titration method. The fractional inhibitory concentration (FIC) index is determined to evaluate the combination effects, which can be interpreted as follows: FIC＝(MIC of compound **A** combined/MIC of compound **A** alone)＋(MIC of compound **B** combined/MIC of compound **B** alone). FIC index≤1 represents synergistic effect, FIC index＞1 and ＜2 represents additive effect, FIC index＞2 represents antagonistic effect.[20,21]

The results of antibacterial activity revealed that some target compounds showed moderate to good antibacterial activities against the tested strains. As depicted in Table 1, the substituents attached on the carbonyl benzene ring exerted different effects on biological activity, and they contributed to the antibacterial activitiy in the order of chloro＞fluoro≈methyl. Among the dichlorophenyl derivatives **5a**－**5e**, compound **5c** gave remarkable inhibitory activity toward *M. luteus* at quite a low concentration (MIC＝0.001 μmol/mL), being 20- and 3-fold more potent than Chloromycin and Norfloxacin, respectively. The anti-*S. aureus* activity (MIC＝0.009 μmol/mL) of derivative **5e** was superior to that of reference drug Chloromycin (MIC＝0.02 μmol/mL) and Norfloxacin (MIC＝0.03 μmol/mL). Additionally, compound **5h** with difluorophenyl moiety was found to possess equivalent antibacterial activity against *M. luteus* (MIC＝0.02 μmol/mL) to Chloromycin. For the dimethylphenyl derivatives, compound **5i** also showed comparable inhibition against *E. coli* (MIC＝0.02 μmol/mL) strain to Chloromycin. Notably, compound **5k** exerted the strongest anti-MRSA activity with MIC vaule of 0.04 μmol/mL, better than Chloromycin (MIC ＝0.05 μmol/mL).

The antibacterial data of compounds **6a**－**6c** implied that the structural modification of the benzotriazole ring at different positions also significantly influenced the biological activity. The *N*-2 modified derivative **6a** with difluorophenyl group exhibited relatively stronger antibacterial activities than other target compounds. *B. proteus* was sensitive to compound **6a** with MIC value of 0.02 μmol/mL, which was 5-fold more active than Chloromycin (MIC＝0.10 μmol/mL). In addition, compound **6a** also displayed good activity against *E. coli* with MIC value of 0.02 μmol/mL. It was observed that the introduction of piperidyl group resulted in weak antibacterial activities for compounds **5m**－**5n** and **6c**, suggesting that the piperidyl moiety was unfavorable for this type of compounds in exerting antibacterial efficacy. The better inhibitory activity observed for compounds **5a**, **5i** and **6a** indicated that the unsubstituted ethenyl benzene ring was beneficial for inhibitory potency, and the incorporation of fluoro or dimethylamino moiety in compounds **5b**, **5d**, **5g** and **5l** resulted in the reduced antibacterial efficiency.

The *in vitro* antifungal evaluation displayed that some target compounds were more sensitive to the tested fungi in comparison with bacterial strains, such as compound **6b**. The *N*-1 derived benzotriazole **5e** possessed superior anti-*C. utilis* (MIC＝0.009 μmol/mL) efficiency to Fluconazole (MIC＝0.03 μmol/mL). The *N*-2 modified benzotriazoles **6a** and **6b** exerted better antifungal activities than their corresponding *N*-1 isomers **5f** and **5j**. Notably, compound **6a** exhibited broad antifungal spectrum and good activities with MICs between 0.01 and 0.04 μmol/mL. Its antifungal potencies against *C. utilis* (MIC＝0.01 μmol/mL), *S. cerevisiae* (MIC＝0.02 μmol/mL) and *A. flavus* (MIC＝0.02 μmol/mL) were better than that of Fluconazole (MIC＝0.03, 0.05, 0.84 μmol/mL, respectively). Although piperidyl substituted chalcones **5m**－**5n** showed fairly weak antifungal activity towards majority of the tested fungi, compound **6c** gave strong inhibitory ability (MIC ＝0.005 μmol/mL) against *C. mycoderma*, which was 2-fold more potent than Fluconazole.

The highly bioactive compounds **5e** and **6a** were further investigated for their combination effects with clinical antibacterial Chloromycin and Norfloxacin and antifungal Fluconazole respectively. The FIC index revealed that the main actions of these combined tests were synergistic. As shown in Tables 2 and 3, the tested bacteria in drug combinations were more susceptible towards **5e**, **6a** and reference drugs in contrast with their individual use. The best effect was the combination application of **5e** with Norfloxacin against *B. proteus* with excellent synergism (FIC＝0.125). To our surprise, MRSA exhibited high tolerance to **5e** (MIC＝1.17 μmol/mL) and **6a** (MIC＝0.71 μmol/mL) individually, but suffered increased degree of susceptibility to them in drug combination studies. These results suggested that compounds **5e** and **6a** might be applied in drug combination therapy to overcome drug resistance or toxicity caused by large dosage. Table 4 showed the antifungal combination effects of **5e** and **6a** with Fluconazole. The dose of compound **5e** was reduced to 1/8-fold initial value against *S. cerevisiae* when combined with 1/4-fold original dose of Fluconazole. These results demonstrated that combined applications could enhance antimicrobial activity, overcome drug resistance and broaden antimicrobial spectra, which might be attributed to the different binding sites of these compounds towards the tested microorganism.[22] Furthermore, the availability of this type of drug combinations

still requires investigations to maximize the antimicrobial efficiencies, and deep mechanistic study is required to elucidate the synergistic action.

### Interactions with calf thymus DNA

Deoxyribonucleic acid (DNA) is the main cellular target for many small molecules of biological im-

**Table 2** Combination effects of compounds **5e** and **6a** with antibacterial Norfloxacina

| Bacteria | Compd | MIC | FIC index | Effect | Compd | MIC | FIC index | Effect |
|---|---|---|---|---|---|---|---|---|
| S. A | Norfloxacin | 4 | 1.500 | Additive | Norfloxacin | 1 | 1.125 | Additive |
| | **5e** | 4 | | | **6a** | 16 | | |
| MRSA | Norfloxacin | 0.125 | 0.250 | Synergistic | Norfloxacin | 0.25 | 0.375 | Synergistic |
| | **5e** | 64 | | | **6a** | 32 | | |
| B. S | Norfloxacin | 0.5 | 0.375 | Synergistic | Norfloxacin | 1 | 0.625 | Synergistic |
| | **5e** | 16 | | | **6a** | 64 | | |
| M. L | Norfloxacin | 0.5 | 0.625 | Synergistic | Norfloxacin | 0.5 | 0.750 | Synergistic |
| | **5e** | 2 | | | **6a** | 4 | | |
| B. P | Norfloxacin | 0.5 | 0.125 | Synergistic | Norfloxacin | 0.5 | 0.500 | Synergistic |
| | **5e** | 32 | | | **6a** | 2 | | |
| E. C | Norfloxacin | 0.125 | 0.250 | Synergistic | Norfloxacin | 0.25 | 0.750 | Synergistic |
| | **5e** | 4 | | | **6a** | 2 | | |
| P. A | Norfloxacin | 0.5 | 0.625 | Synergistic | Norfloxacin | 0.25 | 0.375 | Synergistic |
| | **5e** | 32 | | | **6a** | 2 | | |
| B. T | Norfloxacin | 0.5 | 0.625 | Synergistic | Norfloxacin | 0.25 | 0.500 | Synergistic |
| | **5e** | 16 | | | **6a** | 32 | | |

**Table 3** Combination effects of compounds **5e** and **6a** with antibacterial Chloromycin

| Bacteria | Compd | MIC | FIC index | Effect | Compd | MIC | FIC index | Effect |
|---|---|---|---|---|---|---|---|---|
| S. A | Chloromycin | 4 | 0.750 | Synergistic | Chloromycin | 4 | 1.000 | Synergistic |
| | **5e** | 1 | | | **6a** | 8 | | |
| MRSA | Chloromycin | 8 | 0.625 | Synergistic | Chloromycin | 8 | 0.625 | Synergistic |
| | **5e** | 64 | | | **6a** | 32 | | |
| B. S | Chloromycin | 8 | 0.375 | Synergistic | Chloromycin | 16 | 0.625 | Synergistic |
| | **5e** | 16 | | | **6a** | 64 | | |
| M. L | Chloromycin | 1 | 0.250 | Synergistic | Chloromycin | 1 | 0.250 | Synergistic |
| | **5e** | 2 | | | **6a** | 2 | | |
| B. P | Chloromycin | 8 | 0.375 | Synergistic | Chloromycin | 8 | 0.500 | Synergistic |
| | **5e** | 64 | | | **6a** | 2 | | |
| E. C | Chloromycin | 4 | 0.375 | Synergistic | Chloromycin | 8 | 0.625 | Synergistic |
| | **5e** | 4 | | | **6a** | 1 | | |
| P. A | Chloromycin | 4 | 0.375 | Synergistic | Chloromycin | 2 | 0.250 | Synergistic |
| | **5e** | 64 | | | **6a** | 2 | | |
| B. T | Chloromycin | 4 | 0.250 | Synergistic | Chloromycin | 8 | 0.375 | Synergistic |
| | **5e** | 16 | | | **6a** | 16 | | |

**Table 4** Combination effects of compounds **5e** and **6a** with antifungal Fluconazole

| Bacteria | Compd | MIC | FIC index | Effect | Compd | MIC | FIC index | Effect |
|---|---|---|---|---|---|---|---|---|
| C. A | Fluconazole | 0.5 | 1.000 | Synergistic | Fluconazole | 0.5 | 0.625 | Synergistic |
| | **5e** | 32 | | | **6a** | 2 | | |
| C. M | Fluconazole | 2 | 0.625 | Synergistic | Fluconazole | 2 | 0.750 | Synergistic |
| | **5e** | 2 | | | **6a** | 2 | | |
| C. U | Fluconazole | 4 | 0.625 | Synergistic | Fluconazole | 4 | 1.500 | Additive |
| | **5e** | 0.5 | | | **6a** | 4 | | |
| S. C | Fluconazole | 4 | 0.375 | Synergistic | Fluconazole | 8 | 0.625 | Synergistic |
| | **5e** | 8 | | | **6a** | 1 | | |
| A. F | Fluconazole | 32 | 1.125 | Additive | Fluconazole | 128 | 0.750 | Synergistic |
| | **5e** | 8 | | | **6a** | 8 | | |

portance, which has attracted considerable attention in biomedical field for the rational design and construction of new and efficient drugs. Calf thymus DNA is always selected as DNA model because of its medical importance, low cost and ready availability properties.[23] Therefore, the *in vitro* binding studies between biologically active compound **6a** and calf thymus DNA on molecular level were carried out by UV–vis spectroscopy.

Absorption spectroscopy is a useful technique in DNA-binding studies. Generally, hypochromism and hyperchromism are regarded as important spectral features to indicate the change of DNA double-helical structure when bound with small molecules.[24] With a fixed concentration of DNA, UV-vis absorption spectra were recorded with the increasing amount of compound **6a**. As shown in Figure 4, the maximum absorption peak of DNA at 260 nm exhibited proportional increase and slightly red shift with the increasing concentration of compound **6a**. Meanwhile, the absorption value of simply sum of free DNA and free compound **6a** was a little greater than the measured value of **6a**-DNA complex (inset of Figure 4), which indicated that a weak hypochromic effect existed between DNA and compound **6a**. The hypochromic effect along with the slightly red shift preliminarily demonstrated that the action mode might be intercalation.

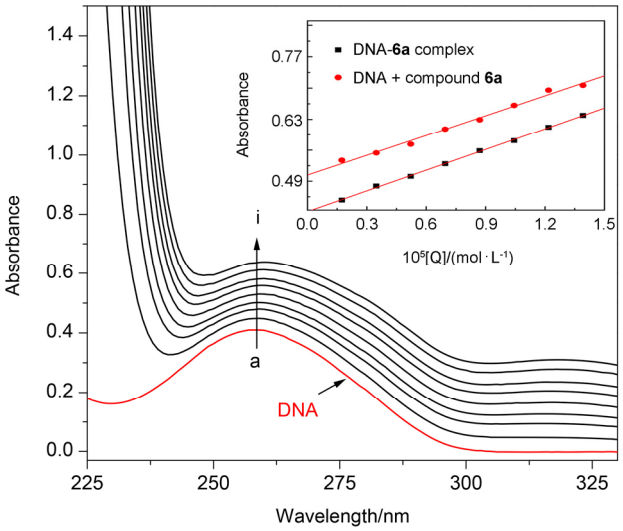



**Figure 4** UV absorption spectra of DNA with different concentrations of compound **6a** (pH=7.4, $T$=303 K). Inset: comparison of absorption at 260 nm between the **6a**-DNA complex and the sum values of free DNA and free compound **6a**. $c$(DNA)=9.78×10$^{-5}$ mol/L, and $c$(compound **6a**)=0−1.39×10$^{-5}$ mol/L for curves a−i respectively at increment of 0.174×10$^{-5}$ mol/L.

On the basis of variations in the absorption spectra of DNA upon binding to **6a**, equation (1)[25] can be utilized to calculate the binding constant ($K$).

$$\frac{A^0}{A-A^0}=\frac{\xi_C}{\xi_{D-C}-\xi_C}+\frac{\xi_C}{\xi_{D-C}-\xi_C}\times\frac{1}{K[Q]} \quad (1)$$

where $A^0$ and $A$ represent the absorbance of DNA in the absence and presence of compound **6a** at 260 nm, $\xi_C$ and $\xi_{D-C}$ are the absorption coefficients of compound **6a** and **6a**-DNA complex respectively. The plot of $A^0/(A-A^0)$ versus 1/[compound **6a**] was constructed by using the absorption titration data and linear fitting (Figure 5), yielding the binding constant, $K$=4.41×10$^{-5}$ L/mol, $R$=0.998, SD=0.21 ($R$ is the correlation coefficient. SD is standard deviation).

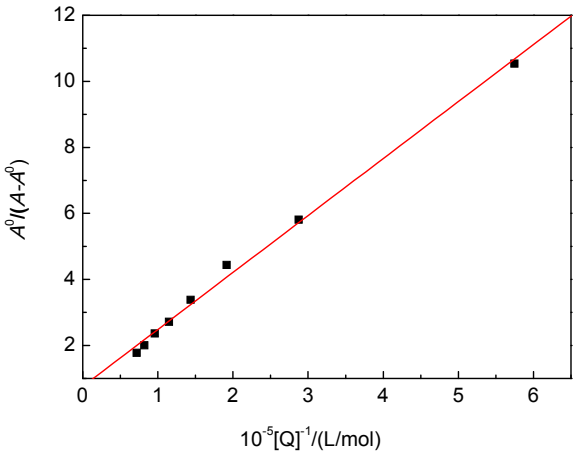



**Figure 5** The plot of $A^0/(A-A^0)$ versus 1/[compound **6a**].

To further explore the action mode of benzotriazolyl chalcone **6a** and DNA, the absorption spectra of the competitive interaction of compound **6a** were also investigated. Neutral red (NR) is a planar phenazine dye with lower toxicity, higher stability and convenient application, and its binding mode with DNA has been confirmed to be intercalative. Therefore, it is always employed as a spectral probe to investigate the binding mode of small molecule with DNA.[26] The absorption spectra of NR upon the addition of DNA were shown in Figure 6. The absorption peak of NR around 460 nm showed gradual decrease with the increasing concentra-

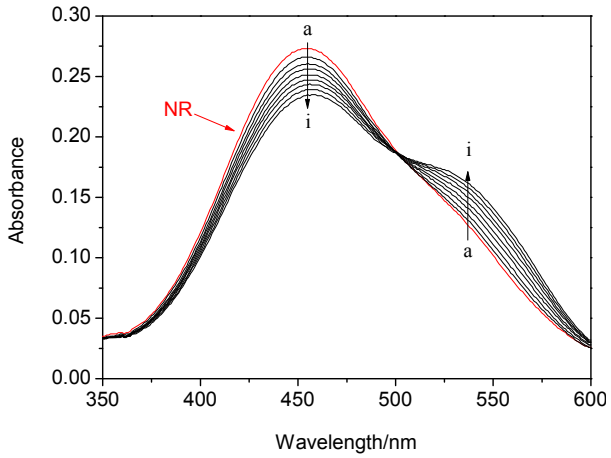



**Figure 6** UV absorption spectra of NR with different concentrations of calf thymus DNA. pH=7.4, $T$=03 K, $c$(NR)=2×10$^{-5}$ mol/L, and $c$(DNA)=0−3.84×10$^{-5}$ mol/L for curves a–i respectively at increment of 0.48×10$^{-5}$ mol/L.

tion of DNA and a new band around 530 nm developed which suggested the formation of the new DNA–NR complex. The isosbestic point at 500 nm provided evidence of the DNA-NR complex formation.

As shown in Figure 7, the competitive binding between NR and **6a** with DNA was observed in the absorption spectra. With the increasing concentration of compound **6a**, an apparent intensity increase was observed around 460 nm. Compared with the absorption around 460 nm of free NR in the presence of the increasing concentration of DNA (Figure 6), the absorbance at the same wavelength exhibited the reverse process (inset of Figure 7). The results suggested that compound **6a** intercalated into the double helix of DNA by substituting of NR in the DNA-NR complex.

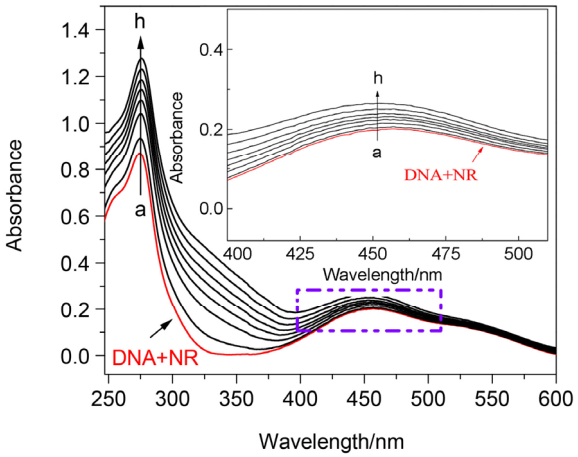



**Figure 7** UV absorption spectra of competitive interaction of compound **6a** and NR with DNA. $c$(DNA)=3.91×10$^{-5}$ mol/L, $c$(NR)=2×10$^{-5}$ mol/L, and $c$(compound **6a**)=0−1.8×10$^{-5}$ mol/L for the curves. (Inset) Absorption spectra of the system with the increasing concentration of **6a** in the wavelength range of 400−510 nm absorption spectra of competitive reaction between compound **6a** and NR with DNA.

## Electronic effects

Literature has reported that electronic effects can reflect the ability of biomolecules to control the pharmacological activities. At molecular level, frontier molecular orbitals (FMO), namely the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) are able to dominate the reactivity of molecule.[27] The results in Figure 8 illustrated that the HOMO of **6a** was mainly located in benzotriazole ring, indicating the existence of possible reactive sites, therefore, electrophilic attacks might take place on these sites; On the other hand, the LUMO of **6a** was primarily concentrated on chalcone backbone in which the negatively charged polar residues of the receptor were favorable. It was remarkable that the low energy gap ($\Delta E$=3.83 eV) of compound **6a** could result in large stabilizing interactions to bind with the receptor, and this result was also evidenced by the value of binding constant ($K$=4.41×10$^4$ L/mol) with calf thymus DNA.[28]

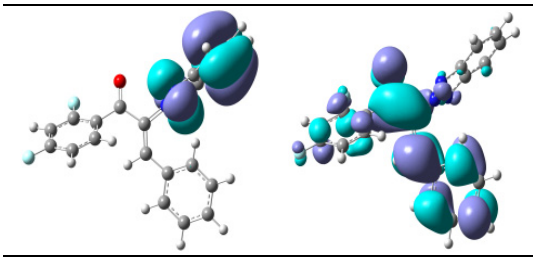



**Figure 8** Plots of the HOMO and LUMO of molecule **6a**.

The molecular electrostatic potential (MEP) surface could give an indication of the charged surface area, probing key structural features for the compounds such as steric, electrostatic interactions and hydrogen donor/acceptor properties.[29]

As shown in Figure 9, the negative charge regions (in red) of compound **6a** mainly located on the *O* atom of carbonyl group and *N* atom of benzotriazolyl moiety, especially on *O* atom, which indicated the strong capability for them to interact with positively charged polar residues of enzymes or receptors.

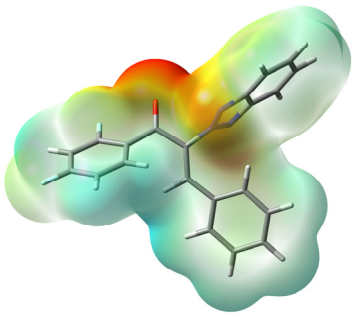


**Figure 9** Molecular electrostatic potentials (MEPs) of compound **6a**.

## Molecular modeling

To rationalize the observed antibacterial activity and to understand the possible mechanism of this type of conjugates, docking studies were carried out. The crystal structure data (DNA gyrase) was obtained from the Protein Data Bank (PDB ID 1Zi0), which was a representative target to investigate the antibacterial mechanism. Active target compound **6a** was selected to dock into the DNA gyrase to rationalize the possible antibacterial mechanism.

The docking result of compound **6a** into the DNA gyrase was shown in Figure 10. The carbonyl group of this molecule was in close proximity to the residues ILE634 and ARG580 through hydrogen bonds, which might correlate with its MEP effect. Moreover, molecule **6a** could also form hydrogen bonds with DNA gyrase through the nitrogen atom of benzotriazolyl moiety and the fluorine atom at 2-position of phenyl group. The latter observation suggested the importance of fluorine atom at 2-position of phenyl group on the antimicrobial activity for that replacing it into piperidyl moiety

(compound **6c**) displayed significantly decrease in bioactivity. In addition, hydrophobic interactions existed between the aromatic ring of compound **6a** and ARG580, ILE634, ALA633 in DNA gyrase. These noncovalent bonds might be beneficial to stabilize compound **6a** in the active sites of DNA gyrase, which might be the crucial reason that compound **6a** displayed strong inhibitory efficacy against some tested strains.

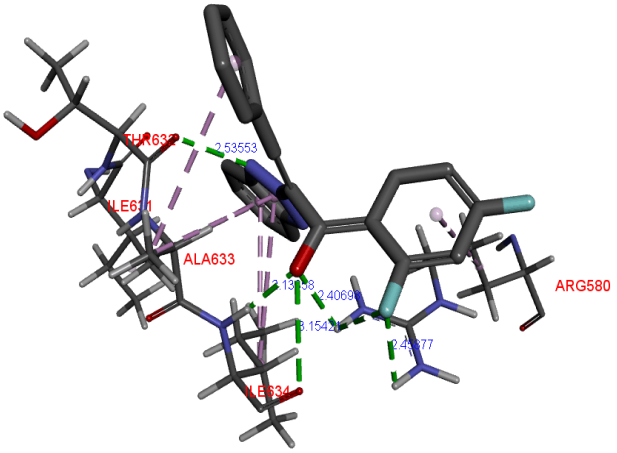



**Figure 10** Three-dimensional conformation of compound **6a** docked in bacterial DNA gyrase.

## Conclusions

In conclusion, a new class of $\alpha$-benzotriazolyl chalcones were successfully synthesized and identified. The antimicrobial assay demonstrated that some target compounds showed moderate to good bioactivities against the tested strains. Particularly, compound **5c** was found to be the most potential one against *M. luteus* with MIC value of 0.001 μmol/mL. Compounds **5e** and **6a** gave superior antibacterial and antifungal activities to their positive controls for some strains. Moreover, most drug combination data of compounds **5e** and **6a** with Chloromycin, Norfloxacin and Fluconazole showed better antimicrobial efficiency with less dosage and broader spectrum than their separated use. Notably, MRSA was highly sensitive in drug combination studies. Meanwhile, the mechanism of interaction of compound **6a** with calf thymus DNA was originally discussed as intercalation which might be a factor to exert its antimicrobial activity. Molecular docking study indicated that compound **6a** could bind with DNA gyrase through hydrogen bonds and hydrophobic interactions. Computational outcomes of active molecule **6a** were accordant with the experimental results to some degree. These observations revealed that this type of benzotriazolyl chalcone scaffold could be recognized as an attractive lead structure for developing novel class of antimicrobial agents. Further studies such as crashing structure-activity relationships discussion, accurately antimicrobial action mechanism exploration, toxicity investigation as well as *in vivo* antibacterial and antifungal evaluation are currently underway in our laboratory.

## Acknowledgement

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