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Synthesis and antimicrobial activity of novel chalcone derivatives

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Abstract A series of novel 3-[*N*, *N*-bis(2-hydroxyethyl)-amino]-chalcone derivatives **3a–3j** were synthesized by the aldol condensation of [*N*, *N*-bis(2-hydroethyl)-3-amino]-acetophenone **2** with aromatic aldehydes. Their structures were further confirmed by ESI-HRMS, ^{1}H NMR, IR and elemental analysis. X-ray analysis reveals crystal **3b** is a monoclinic system with $P2_1/n$ space group. The antimicrobial activities of the newly synthesized chalcones in vitro were evaluated and the results indicated that most compounds presented moderate to good antimicrobial activities, especially the antifungal capability. Compounds **3a**, **3d**, **3f** and **3g** revealed obvious potency against *Candida albicans* with MIC values of 32 μg/mL, which were better compared with others.

Keywords Chalcone · Synthesis · Crystal structure · Broth tube dilution method · Antimicrobial activity

Introduction

Chalcones are the main substructures of some natural compounds and important precursors for the biosynthesis of flavonoids. To date, chalcones have been identified in a variety of plant species such as fruits, vegetables, spices, tea and soy-

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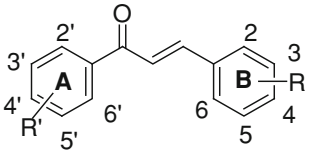


Fig. 1 The basic framework of chalcone

based foodstuffs [1]. The naturally occurring and synthesized chalcone compounds, with a common 1,3-diphenyl-2-propen-1-one framework (Fig. 1), have been reported to possess various pharmacological activities, such as antioxidation [2] anticancer [3], antileishmanial [4], cytotoxicity [5, 6], antituberculosis[7] and antimalarial [8]. Recent reports indicated the importance of chalcones as antiinflammatory and antifungal agents involved in the inhibition of cell migration and the prevention of TNF-α and lipopolysaccharide (LPS) induced neutrophil adhension [9]. Sivakumar et al. [10] found that a coating of chalcone on the polymeric surfaces could reduce the bacterial adhesion due to its bactericidal (cell membrane disruption) [11] activities.

Infectious diseases, such as *Candida albicans* infection, is the major cause of infectious death among patients with chemotherapy-induced myelosuppression neutropenia [12], which is particularly dangerous in people with compromised immune systems such as cancer patients (undergoing immunosuppressive therapy) and AIDS patients. Moreover, increasing numbers of multidrug-resistant microbial pathogens such as methicillin-resistant staphylococcus aureus (MRSA) and vancomycin intermediate-resistant staphylococcus aureus (V-ISA) have made the treatment of infectious diseases a serious and challenging therapeutic problem in recent decades [13]. As pathogenic bacteria and fungi continuously evolve mechanisms of resistance to currently used antibiotics, the discovery and design of novel and potent antibacterial and antifungal drugs are badly needed to overcome microbial resistance and develop effective therapies [14].

With the attempt to search for better and more potential biological activity, we focus on the chalcone derivatives containing different substitutions on one or both of the phenyl rings, which may show potential antifungal and antibacterial properties. Here, we report the kind of newly designed chalcone analogues with *N*-alkyl moiety and different substitutions in a chalcone framework, and the potential antifungal and antibacterial activities of them are also described along with some of their chemical properties.

Experimental

Chemistry and apparatus

Melting points were taken on a XT-4 micro-melting point apparatus and are uncorrected. FT-IR spectra were recorded in KBr disks on an EQUINOX-55 FTIR spectrometer. ^{1}H NMR spectra were run on an INOVA-400 NMR spectrometer with TMS as internal standard. Elemental analyses were carried out on a Vario EL-III

CHNOS analyzer. Electron Spray Ionization-Mass Spectrometry (ESI–MS) analyses were carried out in positive ion modes using a Thermo Finnigan LCQ Advantage MAX LC/MS/MS. Column flash chromatography was carried out on Merck silica gel (250-400 mesh ASTM). The processes of the reactions were monitored by analytical thin-layer chromatography (TLC) using Merck silica gel plates 60 GF-254. Solvents and chemicals used for synthesis were commercially available and were used without further purification unless otherwise noted.

Synthesis

*3-Aminoacetophenone (**1**)*

Iron powder (3.9 g, 60 mL) was activated by refluxing it with the mixture of 10 mL distilled water, ethanol (40 mL), glacial acetic acid (1 mL) and NH_4Cl (0.2 g, 4 mmol) for 0.5 h under vigorous stirring at 80 °C, then ethanol (10 mL) and 3-nitroacetophenone (1.65 g, 10 mmol) were added to the mixture within 10 min. The mixture was then heated slowly under vigorous stirring for 2 h. The hot reaction mixture then filtered and the residue was washed with ethanol 3 times. After evaporation of alcohol, the residue was diluted with water and extracted with V ($CHCl_3$) : V (CH_3OH) = 2:1 for 3 times, the extraction was dried over anhydrous Na_2SO_4 and the solvent was evaporated at reduced pressure. The crude was purified on a flash column [V (CH_3CO_2Et) : V (petroleum ether) = 2:1 as eluent] to give 3-aminoacetophenone **1**. Yield 84 % of white solid. m.p. 96–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.32 (dd, J = 7.6, 0.8 Hz, 1H, H-5), 7.24 (t, J = 7.8 Hz, 2H, H-1 and H-4), 6.89–6.84 (m, 1H, H-3), 3.90 (s, 2H, –NH_2), 2.55 (s, 3H, –CH_3); IR(KBr) ν: 3,467, 3,369, 3,222, 1,668, 1,628, 1,597, 1,490, 1,457, 1,356, 1,324, 1,236, 777, 683 cm^{-1}; Anal. Calc. for C_8H_9NO: C, 71.09; H, 6.7; N, 10.36; Found: C, 70.81; H, 6.73; N, 10.72.

*(E)-[N,N-bis(2-hydroethyl)-3-amino]-acetophenone (**2**)*

A mixture of 3-aminoacetophenone (6.4 g, 47.5 mmol), 2-chlorothanol (16 mL, 240 mmol) and $CaCO_3$ (6.5 g, 65 mmol) in water (60 mL) was heated under reflux with vigorous stirring for 7 h. After hot filtering, the unreacted $CaCO_3$ was washed with few portions of hot water, then the filtrate was extracted with CH_2Cl_2 (40 ml × 3) dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to afford a yellow oil. The residue was further purified by flash column chromatography [V (CH_3CO_2Et) : V (petroleum ether) = 2:1 as eluent] to give the required diol **2**. Yield: 68 % of faint yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.20–7.24 (m, 3H, Ar–H), 6.85–6.87 (m, 1H, Ar–H), 4.74 (s, 2H, –OH), 3.77 (s, 4H, –OCH_2–), 3.53–3.54 (d, J = 4.8 Hz, 4H, –NCH_2–), 2.49 (s, 3H, –CH_3). IR (KBr) ν: 3,378, 2,933, 2,880, 1,673, 1,597, 1,494, 1,444, 1,357, 1,267, 1,178, 1,010, 779, 688 cm^{-1}. HRMS calcd for $C_{12}H_{17}NO_2$: 246.1105 $(M+Na)^+$; Found: 246.1101; Anal. Calc. for $C_{12}H_{17}NO_2$: C, 64.55; H, 7.67; N, 6.27; Found: C, 64.61; H, 7.68; N, 6.26.

General procedure for the preparation of compounds **3a-3i**

To the solution of [*N*, *N*-bis(2-hydroethyl)-3-amino]-acetophenone (3 mmol) and substituted aromatic aldehydes (3 mmol) in ethanol (9 mL) a solution of 2.5 M sodium hydroxide of (1 mL) was added slowly within 10 min in an ice bath. After stirring for 4–6 h at room temperature, the formed precipitate was left. The mixture was extracted with CH_2Cl_2 (10 mL × 3) dried over $MgSO_4$. After the solvent was evaporated under reduced pressure, the crude product was obtained, the crude chalcones was further purified by flash column chromatography SiO_2. The yield, melting point and spectral date of each compound had been collected as below.

(*E*)-*3′-[N,N-Bis(2-hydroxyethyl)-amino-phenyl]-1-phenyl-prop-2-en-1-one* (***3a***) Yield: 87 % of light yellow solid. m.p. 94–96 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.74 (dd, J = 15.7, 2.2 Hz, 1H, H-17), 7.64–7.58 (m, 2H, H-16 and Ar–H), 7.48–7.44 (m, 1H, Ar–H), 7.42–7.38 (m, 3H, Ar–H), 7.31–7.26 (m, 3H, Ar–H), 6.93–6.91 (m, 1H, Ar–H), 4.17 (s, 1H, –OH), 4.02 (s, 1H, –OH), 3.87–3.82 (m, 4H, –OCH_2–), 3.63–3.58 (m, 4H, –NCH_2–). IR (KBr) ν: 3,239, 2,958, 2,926, 1,665, 1,590, 1,489, 1,443, 1,353, 1,320, 1,272, 1,066, 998, 764, 688 cm^{-1}. HRMS calcd for $C_{19}H_{21}NO_3$: 334.1414 $(M+Na)^+$; Found: 334.1407. Anal. Calc. for $C_{19}H_{21}NO_3$: C, 73.29; H, 6.80; N, 4.50; Found: C, 73.36; H, 6.78; N, 4.51.

(*E*)-*3′-[N,N-Bis(2-hydroxyethyl)-amino-phenyl]-1-(4-chlorophenyl)prop-2-en-1-one* (***3b***) Yield: 83.5 % of orange red powder solid. m.p. 115–117 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.69 (d, J = 15.7 Hz, 1H, H-17), 7.55 (d, J = 8.4 Hz, 2H, H-16 and Ar–H), 7.44 (s, 1H, Ar–H), 7.40–7.36 (m, 2H, Ar–H), 7.34–7.27 (m, 3H, Ar–H), 6.94–6.90 (m, 1H, Ar–H), 3.87 (t, J = 4.7 Hz, 4H, –OCH_2–), 3.68 (s, 2H, –OH), 3.63 (t, J = 4.7 Hz, 4H, –NCH_2–). IR(KBr) ν: 3,417, 2,923, 1,655, 1,587, 1,488, 1,446, 1,391, 1,228, 1,072, 815, 770, 727, 680 cm^{-1}. HRMS calcd for $C_{19}H_{20}ClNO_3$: 346.1204 $(M+H)^+$; Found: 346.1201. Anal. Calc. for $C_{19}H_{20}ClNO_3$: C, 65.99; H, 5.83; N, 4.05; Found: C, 66.06; H, 5.81; N, 4.04.

(*E*)-*3′-[N,N-Bis(2-hydroxyethyl)-amino-phenyl]-1-(2-chlorophenyl)prop-2-en-1-one* (***3c***) Yield: Yield: 91 % of deep yellow solid. m.p. 88–90 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.13 (d, J = 15.8 Hz, 1H, H-17), 7.73 (dd, J = 6.8, 2.5 Hz, 1H, H-16) 7.43 (d, J = 15.7, 9.0 Hz, 2H, Ar–H), 7.35–7.28 (m, 5H, Ar–H), 6.92 (d, J = 7.7 Hz, 1H, Ar–H), 3.88 (t, J = 4.8 Hz, 4H, –OCH_2–), 3.83 (s, 2H, –OH), 3.64 (t, J = 4.7 Hz, 4H, –NCH_2–). IR(KBr) ν: 3,415, 3,268, 2,924, 2,867, 1,658, 1,592, 1,496, 1,462, 1,434, 1,391, 1,266, 1,183, 1,082, 749, 684 cm^{-1}. HRMS calcd for $C_{19}H_{20}ClNO_3$: 346.1204 $(M+H)^+$; Found: 346.1197. Anal. Calc. for $C_{19}H_{20}ClNO_3$: C, 65.99; H, 5.83; N 4.05; Found: C, 65.92; H, 5.85; N, 4.06.

(*E*)-*3′-[N,N-Bis(2-hydroxyethyl)-amino-phenyl]-1-(2,4-dichlorophenyl)prop-2-en-1-one* (***3d***) Yield: 82 % of yellow powder solid. m.p. 102–103 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.03 (d, J = 15.8 Hz, 1H, H-17), 7.65 (d, J = 8.5 Hz, 1H, H-16), 7.45 (d, J = 1.9 Hz, 1H, Ar–H), 7.38 (d, J = 15.8 Hz, 1H, Ar–H), 7.33–7.26 (m, 4H, Ar–H), 6.95–6.89 (m, 1H, Ar–H), 3.93 (s, 2H, -OH), 3.86 (t, J = 4.7 Hz, 4H, –OCH_2–), 3.62 (t, J = 4.6 Hz, 4H, –NCH_2–). IR(KBr) ν: 3,251, 2,918, 2,861, 1,660, 1,587, 1,492, 1,465, 1,351, 1,226, 1,081, 1,002, 818, 773, 679 cm^{-1}. HRMS calcd for $C_{19}H_{19}Cl_2NO_3$: 380.0815 $(M+H)^+$; Found: 380.0810. Anal. Calc. for $C_{19}H_{19}Cl_2NO_3$: C, 60.01; H, 5.04; N, 3.68; Found: C, 60.13; H, 5.03; N, 3.66.

(*E*)-*3′-[N,N-Bis(2-hydroxyethyl)-amino-phenyl]-1-(4-bromophenyl)prop-2-en-1-one* (***3e***) Yield: 84 % of orange red lumpish solid. m.p. 121–122 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.66 (d, J = 15.7 Hz, 1H, H-17), 7.53 (d, J = 8.5 Hz, 2H, H-16 and Ar–H), 7.47 (d, J = 8.5 Hz, 2H, Ar–H), 7.42 (d, J = 15.7 Hz, 1H, Ar–H), 7.33–7.26 (m, 3H, Ar–H), 6.90 (dd, J = 7.9, 1.4 Hz, 1H, Ar–H), 4.03 (s, 2H, –OH), 3.85 (s, 4H, –OCH_2–), 3.61 (t, J = 4.8 Hz, 4H, –NCH_2–). IR(KBr) ν: 3,376, 2,922, 1,656, 1,587, 1,486, 1,444, 1,318, 1,231, 1,060, 1,000, 854, 769, 725, 681 cm^{-1}. HRMS calcd for $C_{19}H_{20}BrNO_3$: 390.0699 $(M+H)^+$; Found: 390.0690. Anal. Calc. for $C_{19}H_{20}BrNO_3$: C 58.47; H, 5.17; N, 3.59; Found C, 58.59; H, 5.15; N, 3.58.

(*E*)-*3′-[N,N-Bis(2-hydroxyethyl)-amino-phenyl]-1-(2-fluorophenyl)prop-2-en-1-one* (***3f***) Yield: 91 % of Orange red powder solid. m.p. 80–82 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.82 (d, J = 15.9 Hz, 1H, H-17), 7.60 (m, 1H, H-16), 7.53 (d, J = 15.9 Hz, 1H, Ar–H), 7.39–7.32 (m, 1H, Ar–H), 7.28 (dd, J = 12.7, 4.8 Hz, 3H, Ar–H), 7.17 (t, J = 7.5 Hz, 1H, Ar–H), 7.10 (dd, J = 10.4, 8.7 Hz, 1H, Ar–H), 6.89 (dd, J = 7.4, 2.2 Hz, 1H, Ar–H), 4.46 (s, 2H, –OH), 3.82 (d, J = 4.2 Hz, 4H, –OCH_2–), 3.59 (t, J = 4.8 Hz, 4H, –NCH_2–). IR(KBr) ν: 3,486, 3,376, 2,930, 2,875, 1,652, 1,583, 1,488, 1,450, 1,353, 1,221, 1,067, 1,027, 855, 752 cm^{-1}. HRMS calcd for $C_{19}H_{20}FNO_3$: 330.1500 $(M+H)^+$; Found: 330.1502. Anal. Calc. for $C_{19}H_{20}FNO_3$: C, 69.29; H, 6.12; N, 4.25; Found: C, 69.36; H, 6.10; N, 4.24.

(*E*)-*3′-[N,N-Bis(2-hydroxyethyl)-amino-phenyl]-1-(4-dimethylaminophenyl)prop-2-en-1-one* (***3g***) Yield: 76 % of kermesinus solid. m.p. 106–108 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.73 (d, J = 15.5 Hz, 1H H-17), 7.52 (d, J = 8.3 Hz, 2H, H-16 and Ar–H), 7.25 (5H, t, J = 10.8 Hz, Ar–H), 6.87 (dd, J = 4.3, 2.2 Hz, 1H, Ar–H), 6.68 (d, J = 8.3 Hz, 2H, Ar–H), 3.93 (s, 2H, OH), 3.86 (t, J = 4.4 Hz, 4H, –OCH_2–), 3.61 (t, J = 4.2 Hz, 4H,–NCH_2–), 3.03{s, 6H,–$N(CH_3)_2$}. IR(KBr) ν: 3,421, 2,882, 1,638, 1,606, 1,564, 1,523, 1,439, 1,367, 1,225, 1,166, 1,066, 809, 777, 734, 678 cm^{-1}. HRMS calcd for $C_{21}H_{26}N_2O_3$: 377.1836 $(M+Na)^+$; Found: 377.1835. Anal. Calc. for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90; Found: C, 71.23; H, 7.37; N, 7.88.

(*E*)-*3′-[N,N-Bis(2-hydroxyethyl)-amino-phenyl]-1-(4-fluorophenyl)prop-2-en-1-one* (***3h***) Yield: 89 % of light yellow powder solid. m.p. 103–105 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.70 (d, J = 15.7 Hz, 1H, H-17), 7.60 (dd, J = 7.4, 5.7 Hz, 2H, H-16 and Ar–H), 7.40–7.24 (m, 4H, ArH), 7.09 (t, J = 8.1 Hz, 2H, Ar–H), 6.90 (d, J = 7.6 Hz, 1H, H-2), 4.20 (s, 2H, –OH), 3.84 (t, J = 4.5 Hz, 4H, –OCH_2–), 3.6–3.55 (m, 4H, –NCH_2–). IR(KBr) ν: 3,247, 2,927, 2,871, 1,661, 1,589, 1,502, 1,440, 1,354, 1,223, 1,184, 1,067, 1,002, 830, 783, 699 cm^{-1}. HRMS calcd for $C_{19}H_{20}FNO_3$: 330.1500 $(M+H)^+$; Found: 330.1503. Anal. Calc. for $C_{19}H_{20}FNO_3$: C 69.29, H 6.12, N 4.25; Found: C 69.42, H 6.10, N 4.24.

(*E*)-*3′-[N,N-Bis(2-hydroxyethyl)-amino-phenyl]-1-(4-nitrophenyl)prop-2-en-1-one* (***3i***) Yield: 85 % of kermesinus solid. m.p. 164–166 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.87 (d, J = 8.4 Hz, 2H, H-17 and H-16), 7.77 (d, J = 15.4 Hz, 1H, Ar–H), 7.59 (dd, J = 25.3, 8.6 Hz, 3H, Ar–H), 7.30 (d, J = 15.5 Hz, 1H, Ar–H), 6.70 (d, J = 8.8 Hz, 2H, Ar–H), 3.86-3.78 (m, 4H, –OCH_2–), 3.7-3.63 (m, 4H, –NCH_2–). IR(KBr) ν: 3,424, 2,925, 1,655, 1,590, 1,513, 1,497, 1,448, 1,387, 1,232, 1,103, 1,052, 840, 777, 747 cm^{-1}. HRMS calcd for $C_{19}H_{20}N_2O_5$: 379.1264 $(M+Na)^+$;

Found: 379.1262. Anal. Calc. for $C_{19}H_{20}N_2O_5$: C 64.04, H 5.66, N 7.86; Found: C 64.10, H 5.65, N 7.84.

(*E*)-*3′-[N,N-Bis(2-hydroxyethyl)-amino-phenyl]-1-(2-sulfophenyl)prop-2-en-1-one* (***3j***) To the solution of [*N*, *N*-bis(2-hydroethyl)-3-amino]-acetophenone (3 mmol) and 2-formylbenzenesulfonic acid sodium salt (3 mmol) in ethanol (9 mL) a solution of 2.5 M sodium hydroxide of (1 mL) was added slowly within 10 min in an ice bath. After stirring for 4–6 h at room temperature, the reactant was poured into water, then 2 M HCl was added to precipitate the product. The crude solid was recrystallized from water, giving a gray solid. Yield: 87 %. m.p. 243–245 °C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 8.77 (d, $J = 14.7$ Hz, 1H, H-17), 7.98–7.70 (m, 3H, Ar–H), 7.47 (d, $J = 39.0$ Hz, 5H, Ar–H), 6.01 (s, 4H, –OH, and Ar–H), 3.64 (s, 4H, –OCH_2–), 3.54 (s, 4H, –NCH_2–). IR(KBr) ν: 3,364, 3,037, 2,949, 1,658, 1,582, 1,466, 1,380, 1,237, 1,169, 1,079, 1,018, 862, 758, 710, 615 cm^{-1}. HRMS calcd for $C_{19}H_{21}NSO_6$: 390.1005(M–H)$^-$; Found: 390.1022. Anal. Calcd for $C_{19}H_{21}NSO_6$: C, 55.20; H, 4.88; N, 3.41; Found: C, 55.34; H, 4.87; N, 3.41.

Pharmacology

The following micro-organisms were used for detecting antimicrobial activity: *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Pseudomonas aeruginosa* ATCC 27853, *Serratia typhi* ATCC 14028 and *Escherichia coli* ATCC 25922. All were obtained from the Institute of Microbiology of Shaanxi province.

The preliminary screening for antibacterial as well as antifungal activity of the ten tested compounds in DMSO (25 mg/mL) was evaluated using plate-hole diffusion assay [15, 16]. A twofold serial dilution technique [17] was used to determine the minimum inhibitory concentration (MIC) values of the screened compounds. The MIC value was considered as quantitative method and was used for evaluation of the antimicrobial activity of the synthesized compounds against the given microbes.

Preliminary screening for antimicrobial activity

Suspensions of the above-mentioned micro-organisms were prepared by inoculating fresh stock cultures into separate broth tubes, each containing (8 mL) of Muller–Hinton broth for colonies of bacterial strains and fungal strains. The inoculated tubes were incubated at 37 and 28 °C for 24 h, for bacterial and fungal strains, respectively. Then, 250 μL of bacterial culture ($\sim 10^6$–10^8 bacteria every mL) was added aseptically to 20 mL of solid culture (a mixture of Tryptone, Sabouraud dextrose agar and Yeast Extract) at 45 °C, mixed well, and poured into a sterile Petri dish (90 mm). A sterile cork-borer of 6 mm diameter was used to make 2 wells on each of the Petri dish. One was filled with 10 μL of the screened compounds. In order to check the effect of the solvent, a control test was also performed containing only DMSO. Then the cultures were incubated at 37 and 28 °C for 24 h for the bacterial and fungal strains. After incubation, the zone of inhibition on the plates was recorded. The compounds with the diameter of the growth-inhibition zones

greater than 6 mm were considered positive, and can be screened for further determination of the minimum inhibitory concentration (MIC) values. The test was repeated three times for each compound. All the new compounds (**3a–3j**) were subjected to preliminary biological screening.

Determination of the minimum inhibitory concentration (MIC)

A broth tube dilution method [17, 18] was followed to determined the MIC values for all the screened compounds against the micro-organisms, and for comparison ciprofloxacin was used as a reference. For sample preparation, each of the test compounds and reference were dissolved in DMSO at a concentration of 1,280 μg/mL, and further dilutions of the compounds and reference in culture medium MHB (Müller–Hinton Broth) were prepared at the required quantities of 128, 64, etc., down to 0.125 μg/mL. Bacterial strains and fungal strains were maintained on MHA (Müller–Hinton Agar) medium at 37 and 28 °C for 24 h, respectively. The microbial inocula were prepared by suspension in 10 mL of culture medium for colonies from culture on MHA. The cell density of each inoculum was adjusted in culture medium of a 0.5 McFarland standard. The final amount applied was 10^5 CFU/mL for bacteria and fungi. The microbial inocula were added to the twofold diluted samples. MIC values were read after incubation at 37 and 28 °C for bacterial and fungal strains, respectively. After 24 h, the last tube with no growth of micro-organisms was recorded to represent the MIC value expressed in mg/mL. All experiments were carried out three times.

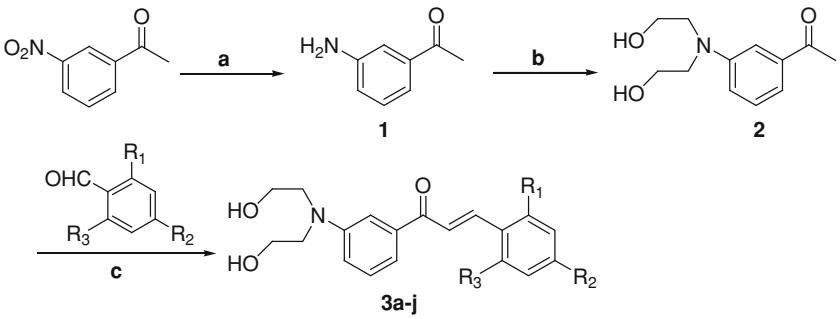
Results and discussion

Synthesis

The *N*-alkoxylated chalcones were synthesized by the base-catalyzed Claisen–Schmidt condensation [19] of 3-[*N*,*N*-bis(2-hydroxyethyl)-amino]-acetophone with appropriate aromatic aldehydes; the synthesis route is illustrated in Scheme 1. Intermediate 3-[*N*,*N*-bis(2-hydroxyethyl)-amino]-acetophone **2** was synthesized through the hydroxyethylation of 3-aminoacetophenone **1** with 2-chlorothanol [20]. While 3-amino-aceophenone **1** was prepared from 3-nitroacetophenone by reduction with Fe/NH_4Cl-CH_3COOH [21]. Ten substitued chalcone derivatives were synthesized, and their structures were confirmed by IR, ^1H NMR, mass spectrometry techniques, and elemental analyses. Crystal of chalcone **3b** was also obtained through the solvents evaporation method, and its X-ray single crystal diffraction data was collected. All the spectral and crystal data are given as supporting information.

Crystal and molecular structure

Chalcones are cross-conjugated molecules, and the carbonyl group in these systems breaks the conjugation system into two independent parts to have a 2D β character



3a: $R_1 = R_2 = R_3 = H$ **3b:** $R_1 = R_3 = H$; $R_2 = Cl$ **3c:** $R_1 = R_2 = H$; $R_3 = Cl$

3d: $R_1 = R_2 = Cl$; $R_3 = H$ **3e:** $R_1 = R_3 = H$; $R_2 = Br$ **3f:** $R_1 = F$; $R_2 = R_3 = H$

3g: $R_1 = R_3 = H$; $R_2 = F$ **3h:** $R_1 = R_3 = H$; $R_2 = N(CH_3)_2$

3i: $R_1 = R_3 = H$; $R_2 = NO_2$ **3j :** $R_2 = R_3 = H$; $R_1 = SO_3H$

Scheme 1 Synthetic scheme for the synthesis of compounds **1, 2, 3a–3j**. Reagents and conditions: *a* Fe, NH_4Cl, CH_3COOH, ethanol, reflux, 2 h; *b* $CaCO_3$, 2-chlorothanol, H_2O, 120 °C, 7 h; *c* 2.5 M NaOH, ethanol, r.t., 3–6 h

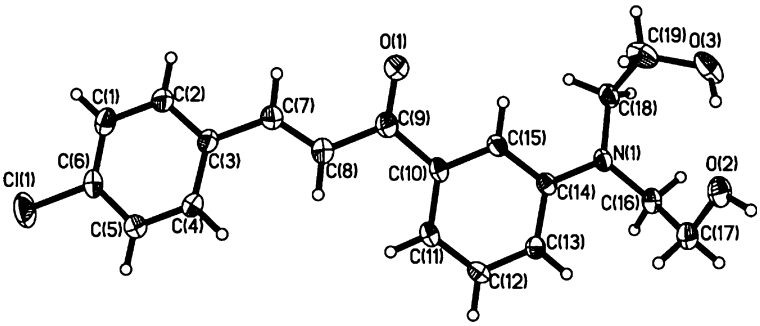


Fig. 2 A molecular drawing of the asymmetric unit of the compound **3b**

[22]. In the molecular structure of chalcone **3b**, a Cl atom and a *N*,*N*-bis(2-hydroxyethyl)-amino group existed in the para position of the B ring and the meta-position of the other, respectively (Fig. 2), and an electron acceptor carbonyl (C=O) group at the middle form a donor-π-accept-π-withdrawing (D-π-A-π-W) system, where charge transfer takes place from the donor end to the withdrawing end. The electron transfer mode may help to align the molecule in a parallel head-to-tail alignment in the crystal packing.

X-ray analysis reveals crystal **3b** is a monoclinic system with an $P2_1/n$ space group. The bond lengths and angles are generally normal in compound **3b**, which is in good agreement with the literature report [23]. The two benzene rings [C3, C2, C4, C5, C6, C1] and [C10, C11, C12, C13, C14, C15] are fairly planar, respectively, and the dihedral angles between the aromatic rings are 27.94°. In the crystal lattice, there are several potentially weak hydrogen bond inter- and intra-molecular interactions [O2–H2···O3, 2.763 Å, 175°, symmetry code i: (x, y, z); O3–H3···O2,

2.720 Å, 165°, symmetry code: (x, y, z); C11–H11···O3, 3.292 Å, 136°, symmetry code: (x, y, z)]. The crystal packing is further stabilized by these hydrogen bonds.

Biological evaluation

Ten of newly synthesized chalcones were evaluated for their antibacterial activity in vitro against Gram-positive bacteria: *S. aureus* and *B. subtilis*; Gram-negative bacteria: *P. aeruginosa*, *E. coli* and *S. typhi*; and anti-fungal activity against *C. albicans*. The results of preliminary biological screening of all the newly synthesized chalcones against the above strains are presented in Table 1. As we can see, most chalcones exhibited moderate antibacterial activity for Gram-positive bacteria (*S. aureus* and *B. subtilis*) and Gram-negative strains (*P. aeruginosa*, *S. typhi* and *E. coli*) except **3b**, **3c**, **3h** and **3i**. Compounds **3d** and **3e** did not show any activity at all to *B. subtilis*. Also, **3e** showed no potency against *E. coli* and *P. aeruginosa*. Interestingly, unexpected results were obtained that all the chalcones revealed moderate to good activity against fungus (*C. albicans*). Thus, we screened all the other compounds except **3b**, **3c**, **3h**, **3j** for further determining of MIC values.

The minimum inhibitory concentration [24] (MIC) values of the screened compounds are outlined in Table 2. Compound **3f** with 2-F in the B ring exhibited a high potency against *C. albicans* and *S. typhi*, with MIC values of 32 and 64 μg/mL, respectively. This compound is also effective in inhibiting the growth of the rest of microorganism. Compounds **3 g** and **3j** possessing 4-F and 2-SO_3H in the B ring revealed good antifungal activity against *C. albicans* with MIC value of 32 μg/mL, while compounds **3e** and **3d** have a lower degree of potency against all the tested microbes. Compound **3a** with no substituent in the B ring showed good antifungal activity, and moderate to obvious antibacterial activity. Compounds **3a, 3g** and **3j**

Table 1 Antimicrobial activity[a] of the newly synthesized chalcones[a] ($C_{chalcones}$ = 25 mg/mL)

Compounds	Fungi	Gram-positive bacteria		Gram-negative bacteria		
	C. albicans	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. typhi*	*E. coli*
3a	+++	++	++	++	++	++
3b	+	−	−	−	−	−
3c	+	++	−	−	−	−
3d	+++	−	++	++	++	−
3e	+	−	++	−	++	−
3f	+++	++	++	++	++	++
3g	+++	++	++	++	++	++
3h	+	−	−	−	−	−
3i	+	−	−	−	−	−
3j	+++	++	++	++	+ +	++

Values were the averages of duplicate experiments

[a] Activity was determined from plate-hole diffusion assays with values to indicate the diameters of zones of growth inhibition around the holes. The diameter of zones of inhibition

+++ >10 mm, ++ 8−10 mm, + 6−8 mm, − <6 mm

Table 2 MIC values of antimicrobial activity of the screened compounds

Compounds	MIC(ug mL^{-1})					
	Fungi	Gram-positive bacteria		Gram- negative bacteria		
	C. albicans	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*S. typhi*	*E. coli*
3a	32	64	128	128	64	128
3d	128	128	Nt[a]	>128	128	Nt
3e	128	128	Nt	Nt	128	Nt
3f	32	128	128	128	64	128
3g	32	128	128	128	128	>128
3j	32	128	128	128	128	>128
Ciprofloxacin[b]	2.00	0.25	0.125	2.00	16	0.125

Minimum inhibitory concentration of the screened compounds were determined using broth tube technique, values were the average ones of duplicate experiments

[a] *Nt* not tested

[b] Ciprofloxacin was used as a control

revealed potent inhibitory effects against *C. albicans*, which were as active as the standard drug (ciprofloxacin) but less active than it. Combining the results of the preliminary biological screening test, it seemed that with substituent as fluorine or no substituent in the B ring, the newly synthesized chalcones showed potent inhibitory effects against both all the tested microbes, especially the fungi. While substituent such as with the chloride, nitro, and dimethylamino groups or bromine in the B ring, the new chalcones revealed less or even no inhibitory effects. And as we have seen, there is no significant difference of antimicrobial activity observed when the electron-withdrawing groups took the place of electron-donating ones on the phenyl ring, and those are consistent with the related report [25]. Thus, it was predicted that the electronic effect of the substituent on the phenyl rings may not be the key factor influencing antimicrobial activity as much as we assumed.

Conclusion

In conclusion, a series of novel 3′-[*N*, *N*-Bis(2-hydroxyethyl)-amino]-chalcone derivatives were synthesized, characterized and evaluated for their antimicrobial activity. The results of preliminary screening of 10 compounds showed that only 6 of them revealed antimicrobial activities, and of those some presented moderate to good potency against the selected microorganisms, especially against *C. albicans*. Compounds **3a**, **3d**, **3f** and **3g** revealed obvious potency against *C. albicans* with MIC value of 32 μg/mL, and this reveals that these compounds may be of potential value in using as antifungal agents. The results may provide us a new prospect of the structure modifications of chalcones derivatives for further biological investigations. On the other hand, more chalcone derivatives of this kind with different substitutions need to be designed and synthesized for further investigation of the analysis of structure–activity relationships (SAR).

X-ray crystallography data

All measurements of compound **3b** was made on a Bruker Smart APEX II CCD diffractometer with graphite monochromated Mo–Ka radiation. The data was collected at a temperature of 296(2) K using the ω-2θ scan technique. Crystal data and structural refinement parameters, and selected bond angles (Å) and bond lengths (°) of **3b** are summarized in Table S1 and Table S2, respectively (see supporting information). CIF files of compound **3b** have been deposited with the Cambridge Structural Database (CCDC No. 886491). Copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html or from the CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK; Tel: (+44) 1223-336-408; fax: (+44) 1223-336-033. E-mail:deposit@ccdc.cam.ac.uk.

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