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Synthesis, Characterization, and In Vitro Antibacterial Activities of Macromolecules Derived from Bis-Chalcone

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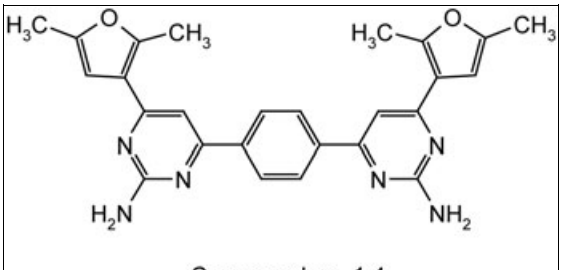

Compound no. 1.4

We investigated the antibacterial activity of some new macromolecules such as bis-pyrazoline, bis-pyrazole, bis-pyrimidines prepared from the reaction of bis-chalcone with thiosemicarbazide/phenyl hydrazine/guanidine hydrochloride/thiourea. All the macromolecules have been characterized by IR, ^{1}H NMR, ^{13}C NMR, mass and elemental analyses. The antibacterial activity of these compounds was first tested *in vitro* by the disc diffusion assay against two Gram-positive and two Gram-negative bacteria, and then the minimum inhibitory concentration was determined with the reference to standard drug chloramphenicol. The results showed that pyrazoline derivative showed better antibacterial activity on *S. typhimurium* and *E. coli* than the reference drug chloramphenicol.

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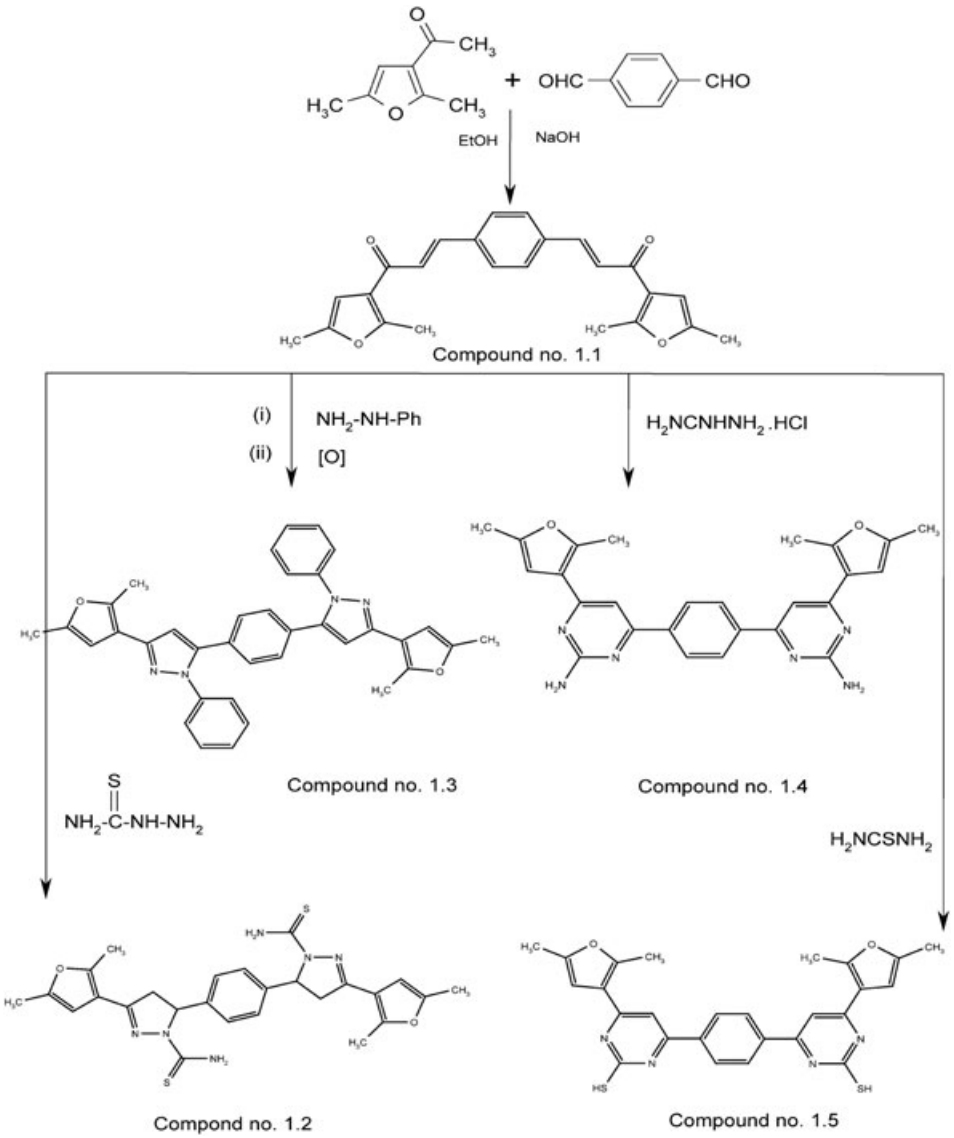
INTRODUCTION

Chalcones (*trans*-1,3-diphenyl-2-propen-1-ones) are carbonyl system; chemically, they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system [1]. The naturally occurring compounds of chalcones belong to a flavonoid family and are present in variety of plant species such as fruits, vegetables, spices, tea, and soy-based foodstuff. Chalcones have been recently focused as a pharmacologically significant group for their interesting biological activities. Chalcones isolated from natural products are known to possess several important activities including antifungal [2], leishmanicidal [3], and antimalarial [4]. Recent reports indicate the importance of chalcones as anti-inflammatory agents involved in inhibition of cell migration and inhibition of TNF synthesis in mouse [5]. Plethora of literature is available describing the role of chalcones and related derivatives such as anticancer [6], anti-inflammatory [7], antimitotic [8], antitubercular [9], cardiovascular [10], and hyperglycemic agents [11]. Chalcones are also used as intermediates for the formation of heterocyclic compounds. Five- and six-member heterocycles are abundant in nature and are of great significance to life because their structural subunits exist in many natural products such as vitamins, hormones, and antibiotics [12]. Hence, they have attracted considerable attention in the design of biologically active molecules [13]. A practical method for the synthesis of such compounds is of great interest in synthetic organic chemistry. Among the heterocycles, pyrazoline and pyrazole are a class of compounds with biological activities, such as antimicrobial [14], antitumor [15], antioxidant [16], calcium channel modulators [17], and antipyretic [18]. On the other hand, the classes of pyrimidines possess a broad spectrum of biological effectiveness such as antitubercular [19], calcium channel blockers [20], and many classes of chemotherapeutic agents containing pyrimidine nucleus are in clinical use. Apart from these, we derived the pyrazoline, pyrazole, and pyrimidines from the chalcone as a antibacterial agents.

RESULTS AND DISCUSSION

Chemistry. In this work, bis-chalcones such as bis-pyrazoline, bis-pyrazole, and bis-pyrimidines were prepared by the reaction of chalcone with In the present work, the cyclization of a bis-chalcone into the corresponding bis-pyrazoline, bis-pyrazole and bis-pyrimidine derivatives was accomplished by the reaction of the chalcone with thiosemicarbazide /phenyl hydrazine/guanidine hydrochloride/thiourea [21–23]. The synthetic route of compounds is outlined in Scheme 1. The chemical structures of the

Scheme 1. The synthesis of the chalcone and their cyclized products.



synthesized compounds were established by spectroscopic (FTIR, ^{1}H NMR, ^{13}C NMR, mass) and elemental analyses.

The IR spectra of compound **1.1** show the characteristic band at 1655 cm^{-1}, which indicates the presence of —C═O group. The IR spectra of compound **1.2** show the characteristic band at 1539 and 1353 cm^{-1}, which indicate the presence of —C═N and C═S group. The IR spectra of compound **1.3** also show the characteristic bands at 1593 and 1495 cm^{-1}, which indicate the presence of —C═C and C═N group. The IR spectra of compound **1.4** shows the characteristic band at 3356 and 1572 cm^{-1}, which indicate the presence of NH_2 and C═N group. The IR spectra of compound **1.5** show the characteristic band at 1180 and 715 cm^{-1}, which indicate the presence of C—N and C—S group. The structure of the chalcone and their cyclized products was further confirmed by ^{1}H NMR spectra, which prove diagnostic tool for the positional elucidation of the proton. Assignments of the signals are based on chemical shift and intensity pattern.

^{1}H NMR spectra of compound **1.1** show two doublets at 7.22 ppm (J = 15.6 Hz) and 7.72 ppm (J = 15.6 Hz), indicating that the ethylene moiety in the enone linkage is in the trans-conformation in the chalcone. ^{1}H NMR spectra of compound **1.2** shows multiplet of —CH_2 at 3.11–5.94 ppm, which confirmed the cyclization of chalcone into in pyrazoline **1.2**. ^{1}H NMR spectra of compound **1.3** show a singlet at 7.75 ppm due to CH═C protons and no peak in the range 3.11–5.94 ppm is observed, indicating that the oxidation of pyrazoline occurred and pyrazoline converted into pyrazole. ^{1}H NMR spectra of compound **1.4** show a sharp singlet at δ 8.17 due to NH_2 protons; they also show a sharp singlet at δ 6.47 due to HC═C, which confirmed

Table 1

Antibacterial activity of chalcone and their cyclized products, positive control chloramphenicol (Chlora.) and negative control (DMSO) measured by the halo zone test (unit, mm).

	Corresponding effect on microorganisms			
Compounds	*S. aureus*	*S. pyogenes*	*S. typhimurium*	*E. coli*
1.1	10.8 ± 0.3	10.2 ± 0.4	10.6 ± 0.3	11.2 ± 0.4
1.2	16.8 ± 0.4	17.6 ± 0.5	18.5 ± 0.5	18.6 ± 0.5
1.3	11.8 ± 0.5	12.3 ± 0.4	12.4 ± 0.5	13.2 ± 0.4
1.4	13.8 ± 0.5	13.5± 0.4	13.1 ± 0.2	14.2 ± 0.4
1.5	15.8 ± 0.2	16.2 ± 0.5	17.5 ± 0.4	15.5± 0.3
Chlora.	17.0 ± 0.5	18.2 ± 0.4	17.2 ± 0.8	20.0 ± 0.2
DMSO	–	–	–	–

the cyclization of chalcone into pyrimidine ring. ^{1}H NMR spectra of compound **1.5** show a sharp singlet at δ 3.31 due to S—H protons; they also show a sharp singlet at δ 7.28 due to HC═C, which confirmed the cyclization of chalcone into pyrimidine ring.

Finally, ^{13}C NMR (600 MHz, $CDCl_3$) spectra of chalcone and their cyclized product were recorded in $CDCl_3$ and spectral signals are in good agreement with the probable structure details of ^{13}C NMR spectra of all compounds and those data are given in Experimental section.

Characteristic peaks were observed in the mass spectra of chalcone and their cyclized products by the molecular ion peak. The ms spectrum of compound **1.5** shows a molecular ion peak (M^+) *m*/*z* 488.

Antimicrobial activity: Disc-diffusion and microdilution assay. The compounds (1.1–1.5) were tested for their antibacterial activities by disc diffusion method using nutrient broth medium [contained (g/L): beef extract 3 g; peptone 5 g; pH 7.0] [24]. The Gram-positive bacteria and Gram-negative bacteria used in this study consisted of *Staphylococcus aureus*, *Streptococcus pyogenes, Salmonella typhimurium*, and *Escherichia coli*. In the disc-diffusion method, sterile paper discs (0.5 mm) impregnated with compound dissolved in dimethylsulfoxide (DMSO) at concentration 100 μg/mL were used. Then, the paper discs impregnated with the solution of the compound tested were placed on the surface of the media inoculated with the microorganism. The plates were incubated at 35°C for 24 h. After incubation, the growth inhibition zones are shown in Table 1. The chalcone and their cyclized products were further checked by minimum inhibitory concentration (MIC) method. The results are presented in Table 2.

EXPERIMENTAL

All the chemicals and solvents used for this work were obtained from Merck (Germany) and Aldrich chemical company Melting points of the synthesized compounds were determined in open-glass capillaries on Stuart-SMP10 melting point apparatus and are uncorrected. IR absorption spectra were recorded on Shimadzu FTIR-8400s using KBr pellets in the range of 4000–400 cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker-Avance-III 600 MHz spectrophotometer using tetramethylsilane (TMS) as an internal standard. The ^{1}H NMR and ^{13}C NMR chemical shifts were reported as parts per million (ppm) downfield from TMS (Me_4Si). The splitting patterns are designated as follows: s, singlet; d, doublet; m, multiplet. Mass spectra were recorded on EI-MS spectrometer. IR, ^{1}H NMR, ^{13}C NMR, and mass spectra were consistent with the assigned structures. Elemental analyses (C, H, N) were done on a CHN rapid analyzer. All the new compounds gave C, H, and N analysis within 0.03% of the theoretical values. Purity of the compounds was checked by thin layer chromatography (TLC) on Merck silica gel 60 F_{254} precoated sheets in chloroform/methanol mixture and spots were developed using iodine vapors/ultraviolet light as visualizing agent.

2*E*,2′*E*-3,3′-(1,4-Phenylene)bis(1-(2,5-dimethylfuran-3-yl) prop-2-en-1-one. A solution of 3-acetyl-2,5-dimethylfuran (2.34 mL, 0.028 mol) and terephthalaldehyde (2 g, 0.014 mol) in ethanolic solution of NaOH (6 g in 10 mL of ethanol) was stirred for 20 h at room temperature. The solution was poured into ice cold water of pH ~2 (pH adjusted by HCl). The solid was separated and dissolved in CH_2Cl_2, washed with saturated solution of $NaHCO_3$, and evaporated to dryness. The residual was recrystallized from methanol/chloroform.

Yield: 78%; mp 182°C; ^{1}H NMR (DMSO-*d*6) (δ/ppm): 7.72 (d, 2H, *J* = 15.6 Hz, C═CH), 7.22 (d, 2H, *J* = 15.6 Hz, CO═CH), 7.63 (s, 4H, Ar—H), 6.34 (s, 2H, thiophene-H), 2.62 (s, CH3), 2.30 (s, CH_3), 2.09 (s, CH_3), 1.93 (s, CH_3); ^{13}C NMR (600 MHz, $CDCl_3$) δ: 185.65, 158.20, 150.17, 141.60, 136.70,

Table 2

Minimum inhibition concentration (MIC) of chalcone and their cyclized products, positive control: chloramphenicol.

	MIC (μg mL^{-1}) compound					
Bacterial strain	**1.1**	**1.2**	**1.3**	**1.4**	**1.5**	Positive control
S. aureus	256	64	128	128	64	32
S. pyogenes	512	32	256	64	64	32
S. typhimurium	256	32	128	64	32	32
E. coli	512	64	256	128	64	32

128.99, 125.00, 122.41, 105.54, 14.53, 13.05; EI-MS *m/z* (rel. int.%): 376 (76) $[M+1]^+$. IR (KBr) *v*max (cm^{-1}): 2956 (C—H), 1655 (C═O), 1567 (C═C); Anal. calc. for $C_{24}H_{22}O_4$: C, 76.99, H, 5.92, O, 17.09; Found: C, 76.95, H, 5.88, O, 19.98.

Synthesis of pyrazoline (1.2) from thiosemicarbazide. A mixture of bis-chalcone (**1.1**) (0.004 mol), thiosemicarbazide (0.009 mol), and NaOH (0.002 mol) in dry ethanol (30 mL) was refluxed at 80°C for 12 h. The progress of reaction was monitored by TLC. After the completion of reaction, the reaction mixture was poured into acidic ice water to ~pH 2 (adjusted by HCl); the obtained precipitated solid was filtered and recrystallized in methanol and the residue obtained was purified by column chromatography (20:80, diethyl ether: petroleum ether) and the obtained solid was crystallized from EtOH to yield pyrazoline.

Yield: 76.5%; mp 250°C; ^{1}H NMR (DMSO-d_6) (δ/ppm): 7.26 (s, 4H, NH_2), 7.17 (s, 4H, Ar—H), 7.14 (s, 2H, thiophene-H), 5.94 (dd, 2H, H_x, J_{XA}= 3.0 Hz, J_{XB} = 3.6 Hz), 3.68 (dd, 2H, H_A, J_{AB} = 3.0 Hz, J_{AX} = 11.4 Hz), 3.11 (dd, 2H, H_B, J_{BA} = 2.4 Hz, J_{BX} = 12.6 Hz), 2.43 (s, CH3), 2.36 (s, CH_3);^{13}C NMR (DMSO-*d*6) (δ/ppm): 175.46 (C═S), 152.39 (C═N), 151.05, 140.96, 126.01 (Ar—C), 62.45 (CH), 44.48 (CH_2), 14.21, 13.19 (CH_3); EI-MS *m/z* (rel. int.%): 522(45) $[M+1]^+$; IR (KBr) ν_{max} (cm^{-1}): 3304 (NH), 2953 (C—H), 1539 (HC═N), 1353 (C═S), 1090 (C—N); Anal. calc. for $C_{26}H_{28}N_6O_2S_2$: C, 59.98, H, 5.42, N, 16.14, Found: 59.95, H, 5.38, N. 16.11.

Synthesis of pyrazole (1.3) from phenyl-hydrazine. A mixture of bis-chalcone (0.004 mol) (1.1) was refluxed with phenyl hydrazine (0.009 mol) in dry EtOH (20 mL) and catalytic amount of glacial acetic acid at 80°C for 8 h. The progress of reaction was monitored by TLC. After completion of the reaction, the solvent was removed under reduced pressure and the residue obtained was purified by column chromatography (20:80, diethyl ether:petroleum ether) and obtained solid was crystallized from EtOH to yield pyrazole 1.3.

Dark yellow solid (chloroform); Yield: 83.8%; mp 198°C; ^{1}H NMR (DMSO-d_6) (δ/ppm): 7. 28 (m, 10H, Ar—H), 6.42 (s, 2H, thiophene-H), 7.75 (s, 2H, C═CH), 5.93 (s, 4H, Ar—H), 2.49 (s, CH3), 2.37 (s, CH_3);^{13}C NMR (DMSO-*d*6) (δ/ppm): 151.88, 148.88, 144.04, 140.53, 136.53, 130.87, 129.27, 128.93, 126.82, 120.14, 113.03, 112.88, 110.54, 106.83, 105.43, 13.84, 13.69; EI-MS *m/z* (rel. int.%): 552(76) $[M+1]^+$; IR (KBr) ν_{max} (cm^{-1}): 3216 (Ar—H), 2918 (C—H), 1593 (C═C), 1495 (HC═N), 1063 (C—N); Anal. calc. for $C_{36}H_{30}N_4O_2$: C, 78.52, H, 5.49, N, 10.17, Found: C, 78.49, H, 5.45, N, 10.14.

Synthesis of pyrimidine (1.4) from guanidine hydrochloride. A mixture of chalcone (**1.1**) (0.004 mol), guanidine hydrochloride (0.009 mol), and sodium methoxide (0.004 mol) in 15 mL DMF was refluxed at 80°C for 30 h. The progress of reaction was monitored by TLC. After the completion of reaction, the reaction mixture was poured into ice water; the obtained precipitated solid was filtered and recrystallized in methanol and the residue obtained was purified by column chromatography (40:50, diethyl ether:petroleum ether) and the obtained solid was crystallized from ethanol.

Yield: 74.68%; mp 284°C; ^{1}H NMR (DMSO-d_6) (δ/ppm): 8.17 (s, 4H, NH_2), 7.17 (s, 4H, Ar—H), 6.47 (s, 2H, Ar—Pym), 7.89 (s, 2H, thiophene-H), 2.57 (s, CH3), 2.13 (s, CH_3);^{13}C NMR (DMSO-*d*6) (δ/ppm): 163.76 (C—NH_2), 162 (C═N), 149 (C—O), 139.49 (C═N), 139.20, 126.95, 119.50, 105.63, 102.98, 40.32, 36.16, 31.55, 29.29, 22.34, 14.34, 13.23; EI-MS *m/z* (rel. int.%): 454(62) $[M+1]^+$; IR (KBr) ν_{max} (cm^{-1}): 3356 (NH_2), 2923 (C—H), 1572 (C═N); Anal. calc. for $C_{26}H_{24}N_6O_2$: C, 69.01, H, 5.35, N, 18.57, Found: C, 68.97, H, 5.31, N, 18.53.

Synthesis of pyrimidine (1.5) from thiourea. A mixture of chalcone (0.004 mol), thiourea (0.009 mol) in 15 mL DMF was refluxed at 80°C for 12 h in the presence of few drops of HCl. The progress of reaction was monitored by TLC. After the completion of reaction, the reaction mixture was poured into ice water; the obtained precipitated solid was filtered and recrystallized in methanol and the residue obtained was purified by column chromatography (40:50, diethyl ether:petroleum ether) and the obtained solid was crystallized from ethanol.

Yield: 72.8%; mp 176°C; ^{1}H NMR (DMSO-d_6) (δ/ppm): 7.75 (dd, 2H, Ar—H), 7.34 (dd, 2H, Ar—H), 7.71 (s, 2H, thiophene-H), 7.28 (s, 2H, Ar—Pym) 3.31 (s, 2H, SH), 2.54 (s, CH_3), 2.38 (s, CH_3); ^{13}C NMR (DMSO-*d*6) (δ/ppm): 185.02 (SH—C═N), 157.49, 149.84, 141.18, 136.41, 128.89, 125.01, 122.29, 105.71, 40.38, 29.33, 14.13, 13.05; EI-MS m/z (rel. int.%): 488 (62) $[M+1]^+$; IR (KBr) ν_{max} cm^{-1}: 2919 (Ar—H), 1655 (C═C), 1567 (C═N), 1180 (C—N), 715 (C—S); Anal. calc. for $C_{26}H_{22}N_4O_2S_2$: C, 64.18, H, 4.56, N, 11.51, Found: C, 64.13, H, 4.52, N, 11.48.

Organism culture and in vitro screening. Antibacterial activity was done by the disc diffusion method with minor modifications. *S. aureus, S. pyogenes, S. typhimurium*, and *E. coli* were subcultured in BHI medium and incubated for 18 h at 37°C, and then the bacterial cells were suspended, according to the McFarland protocol in saline solution to produce a suspension of about 10^{-5} CFU mL^{-1}: 10 μL of this suspension was mixed with 10 mL of sterile antibiotic agar at 40°C and poured onto an agar plate in a laminar flow cabinet. Five paper discs (6.0 mm diameter) were fixed onto nutrient agar plate. Each test compound (1 mg) was dissolved in 100 μL of DMSO to prepare stock solution; from stock solution, different concentrations 10, 20, 25, 50, and 100 μg/μL of each test compound were prepared. These compounds of different concentration were poured over disc plate. Chloramphenicol (30 μg/disc) was used as standard drug (positive control). DMSO-poured disc was used as negative control. The susceptibility of the bacteria to the test compounds was determined by the formation of an inhibitory zone after 18 h of incubation at 36°C. Table 1 reports the inhibition zones (mm) of each compound and the controls. The MIC was evaluated by the macrodilution test using standard inoculums of 10^{-5} CFU mL^{-1}. Serial dilutions of the test compounds, previously dissolved in DMSO were prepared to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 μg/mL. 100 μL of a 24 h old inoculum was added to each tube. The MIC, defined as the lowest concentration of the test compound, which inhibits the visible growth after 18 h, was determined visually after incubation for 18 h, at 37°C, and the results are presented in Table 2. Tests were done using DMSO and chloramphenicol as negative and positive controls.

CONCLUSION

A chalcone was prepared by the reaction of terephthalaldehyde with 3-acetyl-2,5-dimethylfuran. Treatment of this chalcone with thiosemicarbazide/phenyl hydrazine/guanidine hydrochloride/thiourea afforded the corresponding pyrazoline,

pyrazole, and pyrimidine in good yields. The antibacterial activity of these compounds was examined using culture of bacteria and the results showed that the pyrazoline and pyrimidine increased the antibacterial activity. Among the entire five compounds, pyrazoline derivative showed better antibacterial activity on *S. typhimurium* and *E. coli* than the reference drug chloramphenicol.

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