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Microwave Assisted Synthesis of Some Novel Pyrimidine Derivative and Screening for their Biological Activity

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ABSTRACT

A substituted pyrimidine derivatives were synthesized from chalcone of 3-acetyl-2,5-dimethyl thiophene with corresponding active aldehyde in microwave oven. The newly synthesized compounds were characterized by TLC, IR, ^{1}H NMR, ^{13}C NMR spectral analysis. Pyrimidine derivative were screened for their antibacterial activity *in vitro* by the disk diffusion assay against two Gram-positive and two Gram-negative bacteria and then the minimum inhibitory concentration (MIC) was carried with the reference of standard drugs amoxicillin, ampicillin and ciprofloxacin. The pyrimidine derivatives shows better inhibiting action against both types of bacteria (Gram-positive and Gram-negative) compared to amoxicillin, ampicillin and ciprofloxacin standard drugs.

KEYWORDS

Pyrimidine, Thiophene, Chalcone, Antibacterial activity.

INTRODUCTION

Pyrimidine is a constitutive part of RNA and DNA; therefore it imparts considerable pharmaceutical applications [1]. Literature survey shows that pyrimidine exhibits biological activity such as antimicrobial [2-4], anti-analgesic and anti-inflammatory [5,6], antifungal effects [7], anticancer [8], antimalarial [9] and anti-diabetic [10,11]. activities. Due to this it has great scope to synthesize a new pyrimidine derivatives and screening of their antimicrobial properties. Pyrimidine derivatives will be synthesized by cyclization of different chalcone with various compounds like urea, thiourea and guanidine hydrochloride. It is a recent field within the province of heterocyclic chemistry for the last several years because of their ready accessibility and the broad spectrum towards various biological activities [12-15]. As the reality mentioned above and to find out potentially active agents, we have synthesized some new substituted pyrimidine derivative from 2,5-dimethyl-3-acetyl thiophene [16].

The pharmaceutical branch is great collaboration between scientists of various fields with a single aim to give continuously new and effective drugs. An antibacterial is a property of compounds that may kills the bacteria or slows down their growth [17-19]. The antibacterial is also synonymously used as antibiotic. On the basis of their biological action on microorganism antibacterials are divided into two main groups that is bactericidal, which kill bacteria, and bacteriostatic which

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affect on growth of bacteria. The extensive uses of antibiotics are sometimes causes indecent effects in the patients such as hypersensitivity and allergic effects. The structure of the synthesized compound is most important part as biochemical mechanism, physiological action and acute toxicity will depends upon it.

EXPERIMENTAL

All the chemicals and solvents were obtained from commercial suppliers and used as received. Analytical thin-layer chromatography (TLC) was performed on preloaded Merck silica gel plates (60 F_{254}), visualized with a UV-254 lamp and stained with $KMnO_4$. Melting points are uncorrected and were taken in open capillary tubes using paraffin oil bath. IR spectra were recorded on Perkin-Elmer Model 1600 series FTIR instrument. Standard ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded on Varian Mercury spectrometer in DMSO-d_6 as a solvent and chemical shift values are recorded in δ units of relative to tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on LC/MS (ES-API) instrument.

Microwave assisted synthetic procedure

Step-1: Synthesis of α,β-unsaturated carbonyl compounds or chalcone: Equimolar quantity of 3-acetyl-2,5-dimethyl thiophene (0.01 mol) and aromatic aldehyde (0.01 mol) in dry ethanol (20 mL) taken in 100 mL beaker, a catalytic quantity of sodium hydroxide (1-2 pellets) was added and the reaction mixture was irradiated inside the synthetic microwave for 10 min at 210 W. The reaction was monitored by TLC. After the completion of reaction, the whole content was cooled in an ice-bath and the appeared products was filtered, washed and recrystallized from ethanol.

Step-2: (a) Synthesis of pyrimidine derivative from thiourea: Equimolar quantity of chalcone (0.01 mol) and thiourea (0.01 mol) in DMF (30 mL) and 4-5 drop of HCl was irradiated with microwave oven for 20 min at 210 W. The reaction progress was monitored by TLC. After the completion of reaction, the reaction mixture was poured into ice water to give a precipitate which was filtered off and purified by column chromatography; solvent was separated by rotary evaporator. The solid obtained was crystallized from ethanol.

(b) Synthesis of pyrimidine derivative from urea: Equimolar quantity of chalcone (0.01 mol) and urea (0.01 mol) in DMF (30 mL) and 4-5 drop of HCl was irradiated with microwave oven for 10 min at 210 W. The reaction progress was monitored by TLC. After the completion of reaction, the reaction mixture was poured into ice water to give a precipitate that was filtered off and purified by column chromatography; solvent was separated by rotary evaporator. The solid obtained was crystallized from ethanol.

(c) Synthesis of pyrimidine derivative from guanidine hydrochloride: Equimolar quantity of chalcone (0.01 mol) and guanidine hydrochloride (0.01 mol) in DMF (30 mL) and sodium methoxide (0.01 mol) was irradiated with microwave oven for 30 min at 210 W. The reaction progress was monitored by TLC. After the completion of reaction, the reaction mixture was poured into ice water to give a precipitate that was filtered off and purified by column chromatography; solvent was separated by rotary evaporator. The solid obtained was crystallized from ethanol.

4-(4-Chlorophenyl)-6-(2,5-dimethylthiophen-3-yl)-pyrimidine-2-thiol (A): Light-yellow solid: m.p.: 164-166 ºC; IR (KBr, ν_{max}, cm^{-1}): 678 (C-S), 1134 (C-Cl), 1590 (C=N), 1621 (C=C), 2960 (Ar-H); ^{1}H NMR (300 MHz, DMSO-d_6) (δ, ppm): 2.39 (s, -CH_3), 2.48 (s, -CH_3), 3.27 (s, 1H, -SH), 7.11 (s, 1H, thiophene-H), 7.33 (s, 1H, Ar-pyrimidine), 7.51 (d, 2H, Ar-H), 7.65 (d, 2H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 14.47, 14.57, 134.74, 136.21, 142.80, 144.21, 145.61, 149.70, 184.93; EI MS *m/z*: 332.80 (75) [M+1]$^+$; Anal. calcd. (found) % for $C_{16}H_{13}N_2S_2Cl$: C 57.74 (57.68), H 3.93 (3.95), N 8.41 (8.40).

4-(2,5-Dimethylthiophen-3-yl)-6-(4-nitrophenyl)-pyrimidine-2-thiol (B): Light-yellow solid: m.p.: 122-124 ºC; IR (KBr, ν_{max}, cm^{-1}): 678 (C-S), 1535 (N=O), 1590 (C=N), 2930 (C-H), 3020 (Ar-H); ^{1}H NMR (300 MHz, DMSO-d_6) (δ, ppm): 2.39 (s, -CH_3), 2.52 (s, -CH_3), 3.17 (s, 1H, -SH), 7.27 (s, 1H, thiophene-H), 7.39 (s, 1H, Ar-pyrimidine), 7.61 (d, 2H, Ar-H), 7.89 (d, 2H, Ar-H); ^{13}CNMR ($CDCl_3$) δ: 15.05, 16.06, 133.74, 137.21, 140.80, 142.21, 145.51, 148.70, 184.93; EI MS *m/z*: 343.36 (78) [M+1]$^+$; Anal. calcd. (found) % for $C_{16}H_{13}O_2N_3S_2$: C 55.96 (55.92), H 3.81 (3.80), N 12.23 (12.21).

4-(4-methoxyphenyl)-6-(2,5-dimethylthiophen-3-yl)pyrimidine-2-thiol (C): Light-yellow solid: m.p.: 100-102 ºC; IR (KBr, ν_{max}, cm^{-1}): 678 (C-S), 1590 (C=N), 2930 (C-H), 3020 (Ar-H); ^{1}H NMR (300 MHz, DMSO-d_6) (δ, ppm): 2.43 (s, -CH_3), 2.48 (s, -CH_3), 3.19 (s, 1H, -SH), 3.75 (s, 1H, -OCH_3), 7.07 (s, 1H, thiophene-H), 7.22 (s, 1H, Ar-pyrimidine), 7.31 (d, 2H, Ar-H), 7.46 (d, 2H, Ar-H); ^{13}CNMR (DMSO-d_6) δ: 14.58, 14.96, 62.30, 121.32, 124.05, 130.74, 132.20, 136.21, 138.80, 140.21, 144.51, 146.70, 168.26; EI-MS *m/z*: 328.39 (87) [M+1]$^+$; Anal. calcd. (found) % for $C_{17}H_{16}ON_2S_2$: C 62.19 (62.17), H 4.91 (4.90), N 8.53 (8.51).

4-(4-Chlorophenyl)-6-(2,5-dimethylthiophen-3-yl)-pyrimidin-2-amine (D): Pale-yellow solid: m.p.: 126-128 ºC; IR (KBr, ν_{max}, cm^{-1}): 1560 (C=N), 2900 (C-H), 3350 (NH); ^{1}H NMR (300 MHz, DMSO-d_6) (δ, ppm): 2.14 (s, CH_3), 2.41 (s, CH_3), 7.10 (s, 1H, thiophene-H), 7.23 (s, 1H, Ar-pyrimidine), 7.35 (d, 2H, Ar-H), 7.46 (d, 2H, Ar-H), 8.02 (s, 4H, NH); ^{13}C NMR (DMSO-d_6) δ: 15.01, 15.25, 125.32, 126.05, 133.74, 135.20, 137.21, 140.80, 142.21, 145.51, 148.70, 174.93; EI MS *m/z*: 313.74 (65) [M+1]$^+$; Anal. calcd. (found) % for $C_{16}H_{12}N_3SCl$: C 61.25 (61.28), H 3.85 (3.83), N 13.39 (13.40).

4-(2,5-Dimethylthiophen-3-yl)-6-(4-nitrophenyl)-pyrimidine-2-amine (E): Light-yellow solid: m.p.: 128-130 ºC; IR (KBr, ν_{max}, cm^{-1}): 1535 (N=O), 1648 (C=O), 2930 (C-H), 3020 (Ar-H), 3350 (NH); ^{1}H NMR (300 MHz, DMSO-d_6) (δ, ppm): 2.39 (s, -CH_3), 2.52 (s, -CH_3), 3.17 (s, 1H, -SH), 7.27 (s, 1H, thiophene-H), 7.39 (s, 1H, Ar-pyrimidine), 7.61 (d, 2H, Ar-H), 7.89 (d, 2H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 15.05, 16.06, 125.32, 126.05, 133.74, 135.20, 137.21, 140.80, 142.21, 145.51, 148.70, 174.93; EI MS *m/z:* 326.31 (69) [M+1]$^+$; Anal. calcd. (found) % for $C_{16}H_{14}O_2N_4S$: C 58.89 (58.92), H 4.32 (4.31), N 17.16 (17.21).

4-(4-Methoxyphenyl)-6-(2,5-dimethylthiophen-3-yl)pyrimidin-2-amine (F): Light-yellow solid: m.p.: 112-114 ºC; IR (KBr, ν_{max}, cm^{-1}): 1648 (C=O), 2930 (C-H), 3020 (Ar-H), 3350 (NH); ^{1}H NMR (300 MHz, DMSO-d_6) (δ, ppm): 2.39 (s,

-CH_3), 2.52 (s, -CH_3), 3.17 (s, 1H, -SH), 3.85 (s, 1H, -OCH_3), 7.27 (s, 1H, thiophene-H), 7.39 (s, 1H, Ar-pyrimidine), 7.61 (d, 2H, Ar-H), 7.89 (d, 2H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 14.48, 15.06, 65.20, 126.32, 127.05, 133.74, 135.20, 138.21, 140.80, 142.21, 145.51, 148.70, 174.93; EI-MS *m/z:* 311.34 (63) $[M+1]^+$; Anal. calcd. (found) % for $C_{17}H_{17}ON_3S$: C 65.98 (65.94), H 5.50 (5.51), N 13.49 (13.48).

4-(4-Chlorophenyl)-6-(2,5-dimethylthiophen-3-yl)-pyrimidin-2-ol (G): Yellow solid: m.p.: 156-158 ºC; IR (KBr, ν_{max}, cm^{-1}): 678 (C-O), 1134 (C-Cl), 1590 (C=N), 1621 (C=C), 2960 (Ar-H), 3414 (OH); ^{1}H NMR (300 MHz, DMSO-d_6) (δ, ppm): 2.51 (s, -CH_3), 2.68 (s, -CH_3), 3.50 (bs, 1H, -OH), 7.21 (s, 1H, thiophene-H), 7.30 (s, 1H, Ar-pyrimidine), 7.41 (d, 2H, Ar-H), 7.50 (d, 2H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 14.78, 15.28, 135.24, 137.31, 145.30, 147.21, 149.61, 151.70, 177.23; EI-MS *m/z:* 316.74 (68) $[M+1]^+$; Anal. calcd. (found) % for $C_{16}H_{13}ON_2SCl$: C 60.57 (60.62), H 4.13 (4.11), N 8.84 (8.81).

4-(2,5-Dimethylthiophen-3-yl)-6-(4-nitrophenyl)-pyrimidin-2-ol (H): Light-yellow solid: m.p.: 118-120 ºC; IR (KBr, ν_{max}, cm^{-1}): 678 (C-O), 1530 (N=O), 1580 (C=N), 2930 (C-H), 3030 (Ar-H), 3417 (OH); ^{1}H NMR (300 MHz, DMSO-d_6) (δ, ppm): 2.43 (s, -CH_3), 2.62 (s, -CH_3), 3.47 (bs, 1H, -OH), 7.34 (s, 1H, thiophene-H), 7.46 (s, 1H, Ar-pyrimidine), 7.71 (d, 2H, Ar-H), 7.92 (d, 2H, Ar-H); ^{13}CNMR ($CDCl_3$) δ: 15.15, 16.20, 135.47, 139.12, 142.08, 144.21, 149.51, 151.30, 173.65; EI-MS *m/z:* 327.29 (98) $[M+1]^+$; Anal. calcd. (found) % for $C_{16}H_{13}O_3N_3S$: C 58.71 (58.74), H 4.00 (3.98), N 12.83 (12.81).

4-(4-Methoxyphenyl)-6-(2,5-dimethylthiophen-3-yl)-pyrimidin-2-ol (I): Pale-yellow solid: m.p.: 108-110 ºC; IR (KBr, ν_{max}, cm^{-1}): 678 (C-O), 1590 (C=N), 2930 (C-H), 3020 (Ar-H), 3415 (OH); ^{1}H NMR (300 MHz, DMSO-d_6) (δ, ppm): 2.30 (s, -CH_3), 2.47 (s, -CH_3), 3.45 (bs, 1H, -OH), 3.80 (s, 1H, -OCH_3), 7.03 (s, 1H, thiophene-H), 7.33 (s, 1H, Ar-pyrimidine), 7.41 (d, 2H, Ar-H), 7.56 (d, 2H, Ar-H); ^{13}CNMR (DMSO-d_6) δ: 14.85, 15.69, 64.36, 122.23, 124.85, 131.24, 133.20, 136.17, 139.10, 140.21, 144.33, 149.21, 167.40; EI-MS *m/z:* 312.32 (74) $[M+1]^+$; Anal. calcd. (found) % for $C_{17}H_{16}O_2N_2S$: C 65.37 (65.32), H 5.16 (5.15), N 8.96 (8.91).

Antibacterial activity: To check antibacterial activity of the compounds two Gram-positive bacteria *Bacillus subtilis, Staphylococcus aureus* and two Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* were used. About 5 mm sterile disc (HIMEDIA) was used ranging from 10 to 50 µL. To prepare the stock solutions of compounds, 10 mg of each different compound was dissolved in 1 mL of DMSO. From these stock, 10, 20, 30, 40 and 50 µL was added on the sterile discs to get 100, 200, 300, 400 and 500 µg, respectively of the compounds. Then these prepared discs were used for antibacterial activity against the selected bacterial strains.

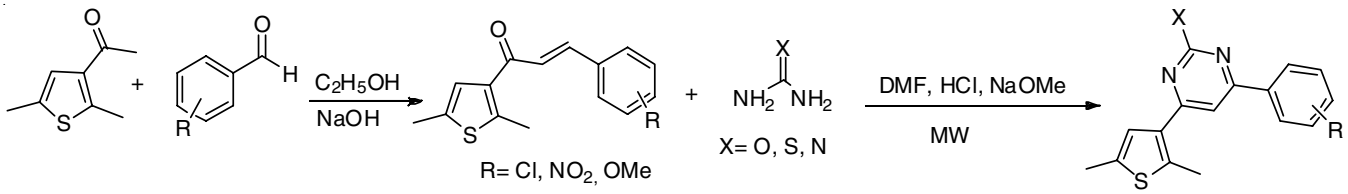
Agar disc diffusion method: Antibacterial activity of all the synthesized compounds were determined by Kirby-Bauer agar disc diffusion method using Muller-Hinton agar medium [20-22]. The sterile cotton swab was immersed into a properly mixed Nutrient broth containing bacterial cultures incubated in the shaker for 8 h at 37 ºC and excess inoculum was removed by pressing the swab against the inner wall of the culture tube. The entire surface of agar plates were swabbed horizontally, vertically and an outer edge of the plate to ensured heavy growth over the entire surface. All the culture plates were allowed to dry for about 5 min. For control results standard antibiotics Amoxicillin (30 µg/disc), ampicillin 25 µg/disc) and ciprofloxacin (30 µg/disc) were used. After the inoculation, each compounds discs as well as antibiotic discs were placed on the separate agar plates using sterile forceps. Then the plates were incubated at 37 ºC for 18-24 h. After the incubation, a clear zone of inhibition around the disc was measured and the results were noted.

RESULTS AND DISCUSSION

Firstly, the chalcones were successfully synthesized from the reaction between 3-acetyl-2,5-dimethyl thiophene with various heterocyclic aromatic aldehydes [23]. The chalcones were reacted with thiourea, urea and guanidine hydrochloride, respectively to form different pyrimidine derivatives according to the known procedure [24]. **Scheme-I** shows the synthetic route for the desired compounds. The reactions proceed in few minutes with good yields in microwave as it has simple and convenient work up procedure. The use of microwave oven is advantages due to required less amount of solvent, less time and high yields (Table-1). The synthesized compounds and their structures were confirmed by spectroscopic data (FT-IR, ^{1}H NMR, ^{13}C NMR, GC-MS).

TABLE-1
COMPARATIVE REACTION TIME AND PERCENTAGE YIELD OF CHALCONE DERIVATIVES (**A-I**) BY CONVENTIONAL AND MICROWAVE IRRADIATION METHODS

Compd.	Time		Energy (W)	Yield (%)
	Conventional method	Microwave method		
A	8 h	10 min	210	78
B	8 h	20 min	210	81
C	8 h	20 min	210	86
D	8 h	10 min	210	78
E	8 h	20 min	210	80
F	8 h	20 min	210	79
G	30 h	30 min	210	83
H	30 h	30 min	210	77
I	30 h	30 min	210	70



Scheme-I

The IR spectra of synthesized compounds **A-C** show a characteristic band at 678 cm^{-1}, which indicates the presence of a C-S group. The IR spectra of all the synthesized compounds **A-I** exhibit the characteristic bands at 1590-1560, 1657-1620 and 1130 cm^{-1}, which indicate the presence of C=N, C=C and C-N group, respectively. Compounds **D-F** also show a characteristic band at 3415 cm^{-1} which indicate the presence of -O-H group, similarly compounds **G-I** show a characteristic band at 3350 cm^{-1} indicates presence of NH group.

The structures of pyrimidine derivative derived from the various chalcones were further predicted by the corresponding ^{1}H NMR spectra. The ^{1}H NMR spectrum of different chalcone compound shows two doublets at 7.10 ppm (J = 15.6Hz) and 7.90 ppm (J = 15.6 Hz), indicating that the presence of *trans*-conformation of ethylene linked with enone. This doublet of chalcones was lost in the ^{1}H NMR spectra of compounds **A-I** which confirmed the cyclization of chalcone into the corresponding pyrimidine derivatives. The ^{1}H NMR spectrum of compound **A** shows a singlet at 3.17 ppm due to S-H proton which also supports to cyclization of chalcones. The ^{1}H NMR spectrum of compound **D** exhibits a broad singlet at 3.47 ppm due to O-H proton, while compound **G** also shows 7.5 ppm confirming the formation of pyrimidine ring. The ^{1}H NMR spectrum of compound **H** showed a sharp singlet at δ 8.04 due to the NH protons which further confirmed the cyclization of the chalcone into a pyrimidine ring. The ^{13}C NMR spectra of the chalcones and pyrimidine derivatives were recorded in DMSO-d_6 and the spectral signals were in good agreement with the on paper structures. The molecular ion peaks were observed in the mass spectra of pyrimidine derivatives strongly supports to the proposed structures.

Antibacterial activity

Compound A: As concentration increases there was increase in zone of inhibition for each bacterium. Higher zone of inhibition for *Bacillus subtilis* was observed at 500 µg concentrations while lower zone of inhibition was observed for 100 µg concentration which was 2.5 mm and 0.5 mm, respectively. For *Staphylococcus aureus*, minimum zone of inhibition concentration was 100 µg and higher zone of inhibition concentration was 500 µg. In case of *E. coli* and *P. aeruginosa* the compound showed good antibacterial activity. A 1.2 mm and 1.6 mm zone of inhibition was observed at 100 µg for *E. coli* and *P. aeruginosa* while 3.1 mm and 2.8 mm zone of inhibition, respectively which was higher one was observed at the concentration of 500 µg (Table-2).

Compound B: Compound **B** shown higher activity against each bacterium at the concentration of 500 µg, higher activity against *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* which was1.8, 1.8, 2.4 and 2.5 mm, respectively for 500 µg concentration and minimum zone of inhibition was 0.4 mm, 0.5 mm, 0.5 mm and 0.7 mm for the concentration of 100 µg (Table-2).

Compound C: Compound **C** shown greater antibacterial activity with higher zone of inhibition 3.1, 4.1, 3.8, 4.1 mm at higher concentration of 500 µg for *B. subtilis*, *S. aureus, E. coli* and *P. aeruginosa*, respectively and lower zone of inhibition 0.8, 0.8, 1.2, 1.6 mm was observed at lower concentration of 100 µg (Table-2).

Compound D: At 500 µg concentration the higher zone of inhibition 3.6 and 3.4 mm was observed against *E. coli* and *S. aureus* and against *B. subtilis* and *P. aeruginosa*, which was 2.8 and 3.3 mm, respectively. The lower zone of inhibition 1.2, 1.0, 1.5 and 1.4 mm was observed, respectively at 100 µg (Table-2).

Compound E: Increase in concentration shows good increase in zone of inhibition against *P. aeruginosa* and *E. coli* 3.7, 3.8 mm and 2.5, 2.8 mm against *B. subtilis* and *S. aureus* at concentration of 500 µg. The minimum zone of inhibition 1.2, 1.2, 1.8, 1.4 mm was observed, respectively at 100 µg (Table-2).

Compound F: The similar higher zone of inhibition 3.9 mm at 500 µg was observed against *P. aeruginosa* and *E. coli* and 2.8 mm, 3.2 mm against *B. subtilis* and *S. aureus*. The minimum zone of inhibition 1.6, 1.2, 1.8 and 1.4 mm was observed against each bacterium, respectively at 100 µg (Table-2).

Compound G: It shows better antibacterial activity against each bacteria. The higher zone of inhibition 4.2, 3.8, 4.5 and 4.2 mm was observed at 500 µg against *Bacillus substilius*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. The minimum zone of inhibition 2.1, 2.2, 2.6, 2.4 mm was observed at 100 µg against *Bacillus substilius*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively.

Compound H: Higher zone of inhibition 5.2 mm at 500 µg was observed against *Escherichia coli* and 3.6, 4.1, 4.3 mm against *Bacillus substilius*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* was observed, respectively. At 100 µg, it shows minimum zone of inhibition 2.2, 2.5, 3.2 and 2.6 mm was observed against *Bacillus substilius*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively.

Compound I: At 500 µg, higher zone of inhibition 5.3 mm was observed against *Escherichia coli* and 2.8, 3.7 and 4.8 mm against *Bacillus substilius*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* was observed, respectively. The minimum zone of inhibition 1.2, 2.2, 3.4 and 2.5 mm was observed against *Bacillus substilius*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively at 100 µg. In case of negative control, no zone of inhibition was observed for positive control, which was used as antibiotics ciprofloxacin has higher zone of inhibition for each bacterium 30.2, 29.4, 32.4 and 34.1 mm for *Bacillus subtilis*, *S. aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Conclusion

A series of substituted pyrimidine derivatives were synthesized by the reaction of chalcone obtained from 3-acetyl-2,5-dimethyl thiophene and corresponding urea, thiourea and guanidine using microwave irradiation method. The antibacterial activity of these synthesized compounds showed that the nitrogen containing heterocyclic pyrimidines possess increased antimicrobial activity. Among the nine synthesized compounds, the compound containing pyrimidine hydroxyl derivatives **H** and **I** exhibited antibacterial activity against *E. coli* better than that of the reference drugs amoxicillin and ciprofloxacin.

TABLE-2
ANTIBACTERIAL ACTIVITY AND MIC OF COMPOUNDS **A-I**

Conc. (µg/disc)	Zone of inhibition against bacteria (mm)							
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
	Compound **A**				Compound **B**			
100	0.5 ± 1.02	0.2 ± 0.12	1.2 ± 0.14	1.6 ± 0.02	0.4 ± 0.24	0.5 ± 1.04	0. 5 ± 1.02	0.7 ± 0.06
200	0.8 ± 0.26	0.6 ± 0.16	1.6 ± 1.04	1.8 ± 1.02	0.6 ± 0.02	0.6 ± 1.12	0.6 ± 0.02	0.9 ± 0.02
300	1.2 ± 0.02	1.1 ± 1.04	2.3 ± 0. 12	2.5 ± 1.02	1.2 ± 1.22	1.5 ± 0.02	1.2 ± 1.04	1.8 ± 1.02
400	1.3 ± 1.12	1.5 ± 1.02	2.8 ± 1.18	2.6 ± 0.06	1.6 ± 1.04	1.6 ± 1.02	1.5 ± 0.02	2.3 ± 0.02
500	2.5 ± 1.02	1.8 ± 0.04	3.1 ± 0.12	2.8 ± 0.02	1.8 ± 0.2	1.8 ± 1.02	2.4 ± 0.02	2.5 ± 1.12
	Compound **C**				Compound **D**			
100	0.8 ± 0.02	0.8 ± 0.02	1.2 ± 0.02	1.6 ± 0.02	1.2 ± 1.02	1.0 ± 1.02	1.5 ± 0.02	1.4 ± 0.02
200	1.5 ± 1.02	1.6 ± 0.2	1.8 ± 0.02	1.8 ± 0.12	1.6 ± 0.04	1.5 ± 1.06	1.9 ± 0.02	1.6 ± 0.02
300	2.1 ± 0.02	2.2 ± 1.02	2.6 ± 1.02	2.5 ± 0.06	2.1 ± 0.02	2.6 ± 1.02	2.3 ± 1.02	2.3 ± 0.02
400	2.6 ± 1.02	3.8 ± 1.02	3.0 ± 1	3.6 ± 0.06	2.2 ± 1.02	2.8 ± 0.02	2.3 ± 1.02	2.6 ± 0.06
500	3.1 ± 1.02	4.1 ± 0.2	3.8 ± 0.02	4.1 ± 0.02	2.8 ± 0.06	3.4 ± 0.02	3.6 ± 1.02	3.3 ± 0.02
	Compound **E**				Compound **F**			
100	1.2 ± 1.02	1.2 ± 0.02	1.8 ± 1.02	1.4 ± 0.2	1.6 ± 0.02	1.2 ± 1.02	1.8 ± 1.02	1.4 ± 0.12
200	1.6 ± 1.02	1.3 ± 0.16	2.2 ± 1.02	1.8 ± 0.16	1.8 ± 1.02	1.6 ± 1.02	2.3 ± 0.02	1.9 ± 0.02
300	1.8 ± 1.02	1.8 ± 0.02	2.6 ± 1.02	2.4 ± 0.12	2.6 ± 1.02	2.6 ± 0.02	2.6 ± 1.06	2.7 ± 1.02
400	2.2 ± 0.02	2.5 ± 1.02	3.4 ± 1.06	2.9 ± 0.02	2.6 ± 0.06	2.9 ± 0.06	3.5 ± 1.06	3.6 ± 1.02
500	2.5 ± 0.02	2.8 ± 1.02	3.8 ± 0.02	3.7 ± 0.02	2.8 ± 1.12	3.2 ± 0.02	3.9 ± 0.02	3.9 ± 1.06
	Compound **G**				Compound **H**			
100	2.1 ± 1.02	2.2 ± 1.06	2.6 ± 1.02	2.4 ± 0.02	2.2 ± 1.02	2.5 ± 0.06	3.2 ± 0.04	2.6 ± 1.02
200	2.2 ± 1.02	2.6 ± 1.02	3.2 ± 0.02	2.6 ± 1.02	2.3 ± 0.12	2.6 ± 1.02	3.9 ± 1.02	2.7 ± 0.06
300	2.8 ± 0.06	2.8 ± 1.02	3.6 ± 0.02	3.3 ± 0.12	2.8 ± 1.02	3.3 ± 0.26	4.3 ± 1.02	3.4 ± 0.02
400	3.6 ± 0.02	3.5 ± 0.04	4.1 ± 0.02	3.9 ± 1.06	3.4 ± 0.16	3.6 ± 0.12	4.6 ± 0.04	3.6 ± 0.06
500	4.2 ± 0.02	3.8 ± 0.02	4.5 ± 0.2	4.2 ± 0.02	3.6 ± 0.2	4.1 ± 0.01	5.2 ± 0.26	4.3 ± 1.02
	Compound **I**							
100	1.2 ± 1.02	2.2 ± 0.04	3.4 ± 1.02	2.5 ± 0.02				
200	1.8 ± 1.02	2.6 ± 0.26	3.6 ± 1.02	3.4 ± 0.06				
300	1.5 ± 0.06	3.2 ± 1.02	4.6 ± 1.02	3.6 ± 0.12				
400	2.6 ± 0.02	3.2 ± 0.26	4.9 ± 0.02	4.2 ± 1.02				
500	2.8 ± 1.02	3.7 ± 0.02	5.3 ± 0.02	4.8 ± 1.02				

	Conc. (µg/disc)	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Negative control	–	–	–	–	–
Amoxicillin	30	23.5 ± 1.05	24.3 ± 0.12	27.9 ± 0.06	26.2 ± 1.04
Ampicillin	25	20.6 ± 0.12	26.1 ± 0.08	25.6 ± 0.14	23.4 ± 1.02
Ciprofloxacin	30	30.2 ± 1.04	29.4 ± 1.02	32.4 ± 0.02	34.1 ± 0.12

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