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Research Article

SYNTHESIS AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF PYRIMIDINE DERIVATIVES

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ABSTRACT

Objectives: Synthesis, characterization, and evaluation of antimicrobial activity of novel pyrimidine derivatives containing O, N, and S in the ring.

Methods: Pyrimidine derivatives were prepared in three steps. In the first step, chalcones containing -NO_2 functional group were synthesized using Claisen-Schmidt condensation of aromatic aldehydes with 2-acetyl pyridine/3-acetylpyridine in methanol in the presence of aqueous NaOH. In the second step, -NO_2 group was reduced to -NH_2 group. Resulting compounds containing NH_2 functional group were reacted with different dichlorothienopyrimidines and dichlorofuropyrimidines in the presence of N,N-diisopropylethylamine to obtain pyrimidine derivatives.Antibacterial and antifungal activity of pyrimidine derivatives were studied *in vitro*.

Results: Pyrimidine derivatives were synthesized and purified using flash column chromatography. Purity of synthesized pyrimidines was determined by high-performance liquid chromatography. Pyrimidines were characterized by elemental analysis, infrared, nuclear magnetic resonance, and mass spectral analysis. Analytical data of synthesized pyrimidines supported the proposed structures. Significant antibacterial and antifungal activity were observed in the synthesized pyrimidine derivatives.

Conclusion: Antibacterial and antifungal activity of the newly synthesized pyrimidine derivatives will definitely inspire future researchers for the preparation of new analogs.

Keywords: 2-Acetylpyridine, 3-Acetylpyridine, Dichlorothienopyrimidines, Dichlorofuropyrimidines, Antibacterial activity and antifungal activity.

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INTRODUCTION

Pyrimidine and its derivatives having two nitrogen atoms at 1 and 3 positions of aromatic ring are found in nature. Pyrimidine derivatives synthesized in laboratory are also reported in literature. Compounds containing pyrimidine ring already attracted significant interest in medicinal chemistry due to their pharmacological and biological applications. Antibacterial [1-4], antifungal [5-7], anti-HIV [8], antitubercular [9], antitumor [10], antineoplastic [11], antileishmanial [12], anti-inflammatory [13-15], antidiabetic [16], antipyretic [17], antioxidant [18,19], antihistaminic [20], anticancer [21,22], and herbicidal [23] properties of pyrimidine derivatives are reported in literatures. Pyrimidine derivatives were also found to possess potential central nervous system-depressant properties [24,25] and to act as blocker of calcium channel [26].

Wide spectrum biological properties of pyrimidine derivatives inspired us to synthesize novel pyrimidine derivatives and to study their antibacterial and antifungal activities.

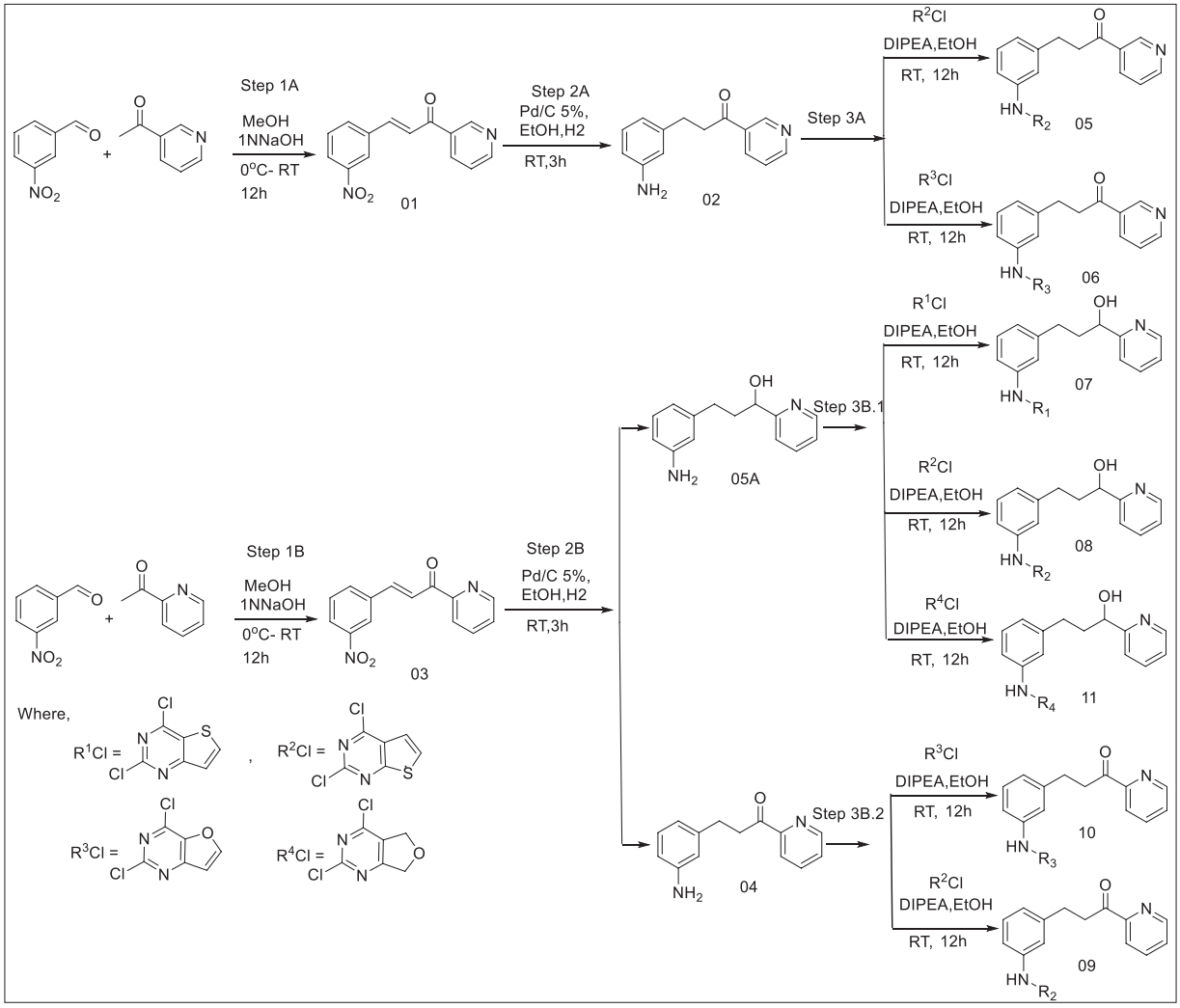
METHODS

LR grade chemicals of Sigma-Aldrich, Alfa Aesar, Combi-Blocks, AK Scientifics, Avra, and SD-Fine Chem Ltd. were used throughout the present investigation. Solvents were dried and distilled before use. Progress of reactions was monitored both by thin-layer chromatography (TLC) using precoated silica plate (TLC Silica gel 60 F_{254}) and high-performance liquid chromatography (HPLC). Flash column chromatography technique using silica gel of mesh size 250–450 was used to purify crudes. Agilent 1260 series HPLC was used to check the purity of pyrimidine derivatives. CHNS analyzer (PerkinElmer 2400) was used for elemental analysis. Molecular ions were determined by mass spectrometer (Agilent Technologies G1946 D). Infrared (IR) spectra were recorded on Agilent Technologies Carry 630. Vibrational frequencies are presented in cm^{-1}. ^1H nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance, DPX 300 using tetramethylsilane as an internal standard and chemical shift values are presented in δ ppm.

Scheme 1 was used to synthesis the pyrimidine derivatives.

Step 1A and Step 1B: General method for the synthesis of chalcones
To a stirred solution of 1-(pyridin-3-yl)ethan-1-one/1-(pyridin-2-yl)ethan-1-one (2.00 g, 16.52 mmol) in 60 mL methanol, 3-nitrobenzaldehyde (2.49 g, 16.52 mmol) was added at 0°C followed by the addition of 3.30 mL of aqueous solution of 1 N NaOH. Resultant reaction mixture was stirred first at 0°C for 30 min and then at room temperature for 12 h. Progress of the reaction was monitored by TLC (mobile phase 80% ethyl acetate [EtOAc] in hexane). The reaction mixture was diluted with 80 mL distilled water and was cooled to 0°C. 10.0 mL 1 N HCl was added to the reaction mixture. Solid was filtered to obtain (E)-3-(3-nitrophenyl)-1-(pyridin-3-yl)prop-2-en-1-one 01 (yield: 3.2 g, 95.23%)/(E)-3-(3-nitrophenyl)-1-(pyridin-2-yl)prop-2-en-1-one 03 (yield: 3.0 g, 89.28%) as an off white solid.

Step 2A and Step 2B: General method for the reduction of -NO_2 group of chalcones to - NH_2 group
Pd/C 5% (0.84 g) was added at room temperature to stirred a solution of (E)-3-(3-nitrophenyl)-1-(pyridin-3-yl)prop-2-en-1-one 01 (1.0 g, 3.94mmol)/(E)-3-(3-nitrophenyl)-1-(pyridin-2-yl) prop-2-en-1-one 03 (1.0 g, 3.94 mmol) in ethanol (40 mL). Air was removed from the reaction flask and hydrogen atmosphere was created by hydrogen balloon. Reaction mixture was stirred for 2 h at room temperature. Progress of reaction was monitored by TLC (mobile phase 5% methanol [MeOH] in dichloromethane [DCM]). Reaction mixture was filtered through celite bed and celite bed was washed twice with EtOH (2×20.0 mL). Filtrate was concentrated and



Scheme 1: Synthesis of pyrimidine derivatives

crude was purified by flash column chromatography using mobile phase (0–5% MeOH in DCM). 3-(3-aminophenyl)-1-(pyridin-3-yl)propan-1-one 2 (yield: 0.5 g, 56.81%) was isolated as a brown solid when (E)-3-(3-nitrophenyl)-1-(pyridin-3-yl)prop-2-en-1-one 1 was used as starting chalcone. Mixture of 3-(3-aminophenyl)-1-(pyridin-2-yl)propan-1-one 4 and 3-(3-aminophenyl)-1-(pyridin-2-yl) propan-1-ol 5A were isolated when (E)-3-(3-nitrophenyl)-1-(pyridin-2-yl)prop-2-en-1-one 3 was used as starting chalcone. Crude was purified by flash column chromatography (mobile phase 0–5% MeOH in DCM) to get title compound 3-(3-aminophenyl)-1-(pyridin-2-yl)propan-1-one 4 (yield: 0.6 g, 68.18%) as a brown solid and 3-(3-aminophenyl)-1-(pyridin-2-yl)propan-1-ol 5A (yield: 0.2 g, 28.08%) as an oily mass.

Step 3A, 3B.1, and 3B.2: General method for the synthesis of pyrimidine derivatives

At room temperature, 2,4-dichlorothieno[2,3-d]pyrimidine/2,4-dichlorothieno[2,3-d]pyrimidine/2,4-dichlorofuro[3,2-d] pyrimidine/2,4-dichloro-5,7-dihydrofuro[3,4-d]pyrimidin(2.65 mmol) and N,N-diisopropylethylamine (DIPEA) (0.853 g, 6.6 mmol) were added to a stirred a solution of 3-(3-aminophenyl)-1-(pyridin-3-yl) propan-1-one 2/3-(3-aminophenyl)-1-(pyridin-2-yl)propan-1-one 4/3-(3-aminophenyl)-1-(pyridin-2-yl)propan-1-ol 5A (2.20 mmol) in ethanol (20 mL). Reaction mixture was stirred at room temperature for 12 h. Progress of reaction was monitored by TLC (mobile phase 0–5% MeOH in DCM). Reaction mixture was concentrated and crude was purified by flash column chromatography (mobile phase 5% MeOH in DCM) to obtain pyrimidine derivatives 5–11 (yield: 20.68, 44.44%) as an off white solid to brown solid.

Antibacterial and antifungal activity

Synthesized pyrimidine derivatives were screened for antibacterial activity against the bacteria *E. coli* and *Bacillus sphaericus* and were screened for antifungal activity against the fungus *Aspergillus niger* and *Penicillium funiculosum.*

Antibacterial assay

In vitro antibacterial activity of synthesized pyrimidine derivatives (in DMSO solution at the concentration of 5 mg/mL) was studied as reported elsewhere [27] against bacterial strains by the agar well diffusion method [28]. Mueller-Hinton agar no. 2 (HiMedia, India) was melted and cooled to 48–50°C. Standardized inoculums (1.5×10^8 CFU/mL, 0.5 McFarland) were added aseptically to the molten agar and were poured into sterile Petri dishes to obtain solid plate. Wells were prepared in the seeded agar plates. The test compound (100 µL) was introduced in the well (6 mm). Plates were incubated overnight at 37°C. Antibacterial spectrum of the test compound was determined for the bacterial species in terms of zone sizes around each well. Diameter of inhibition zone produced by the test compound was compared with that of standard Ciprofloxacin. For each bacterial strain, controls were maintained using pure solvent instead of the sample solution. Control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. Experiments were performed in triplicate.

Study of antifungal assay

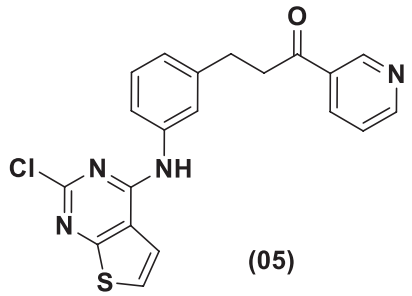
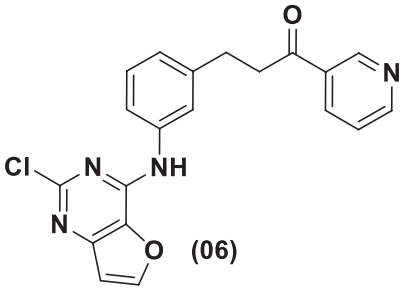
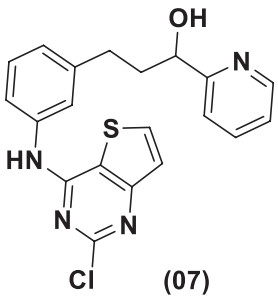
Synthesized pyrimidine derivatives were screened for their antifungal activity as reported elsewhere [27] using agar well diffusion method [29]. The fungal spore was prepared by sterile PBS. Concentration was adjusted to 10^6 cells/mL by dropping by a sterile swab into the fungal suspension and rolled on the surface of the agar medium and plates were dried over 20 min at room temperature. Plates were incubated at 37°C for 36 h. The diameter of inhibition zone (in mm) produced by the subject compound was compared with standard Ketoconazole. Experiments were performed in triplicate.

RESULTS AND DISCUSSION

Purity of the synthesized novel pyrimidine derivatives was determined by HPLC, characterized by elemental analysis, IR, NMR, and mass spectral analysis and was screened for their antibacterial and antifungal activity.

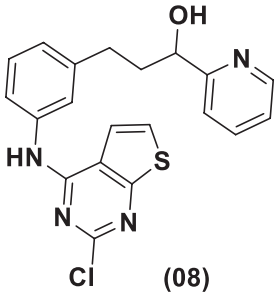
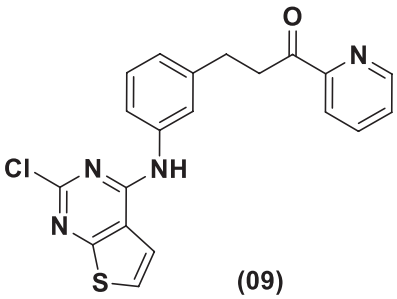
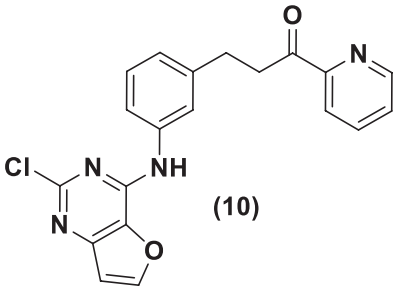
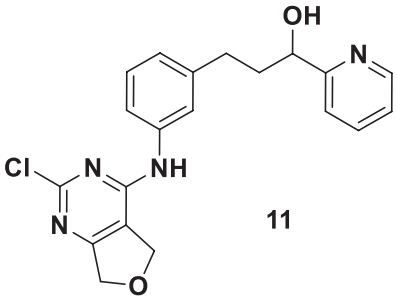
Reaction scheme for the synthesis of pyrimidine derivatives is given in Scheme 1. Compounds 1, 2, 3, 4, and 5A were prepared as reported elsewhere by the authors [25]. In the presence of DIPEA, compounds 3,

Table 1: Elemental analysis, appearance, molecular weight, molecular mass ion, and HPLC purity of pyrimidine derivatives

S. No.	Structure MF MW Appearance	Molecular mass	HPLC purity (%)	Elemental analysis (%) found (calculated)		
				C%	H%	N%
1	 (05) MF: $C_{20}H_{15}ClN_4OS$ MW: 394.88 Off white solid	(M+) 394.9, (M+2) 397.0,	97.88%	60.79 (60.83)	3.84 (3.83)	14.22 (14.19)
2	 (06) MF: $C_{20}H_{15}ClN_4O_2$ MW: 378.82 Brown solid	(M-1) 377.0	98.02%	63.43 (63.41)	4.97 (3.99)	14.81 (14.79)
3	 (07) MF: $C_{20}H_{17}ClN_4OS$ MW: 396.89 Off white solid	397.1(M+) 398.1(M+2)	99.21%	60.51 (60.53)	4.34 (4.32)	14.11 (14.12)

(Contd...)

Table 1: ***(Continued)***

4	 (08) MF: $C_{20}H_{17}ClN_4OS$ MW: 396.89 Off white solid	(M+) 397.0, (M+2) 398.7	99.53%	60.55 (60.53)	4.30 (4.32)	14.09 (14.12)
5	 (09) MF: $C_{20}H_{15}ClN_4OS$ MW: 394.88 Off white solid	(M+) 394.9, (M+2) 397.0	98.09%	60.79 (60.83)	3.82 (3.83)	14.19 (14.19)
6	 (10) MF: $C_{20}H_{15}ClN_4O_2$ MW: 378.82 Brown solid	(M+) 378.8, (M+2) 380.9	96.01%	63.42 (63.41)	4.00 (3.99)	14.80 (14.79)
7	 11 MF: $C_{20}H_{19}ClN_4O_2$ MW: 382.85 Off white solid	(M-1) 381.1	97.84%	62.72 (62.75)	5.01 (5.00)	14.61 (14.63)

HPLC: High-performance liquid chromatography

4, and 5A containing NH_2 functional group were reacted with different dichlorothienopyrimidines and dichlorofuropyrimidines at room temperature for 12 h to undergo condensation reaction resulting to pyrimidine derivatives.

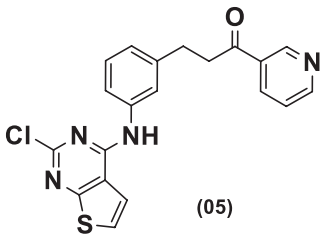
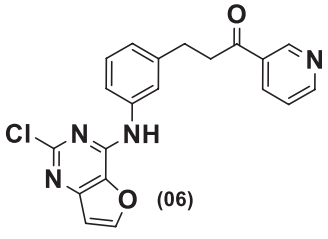
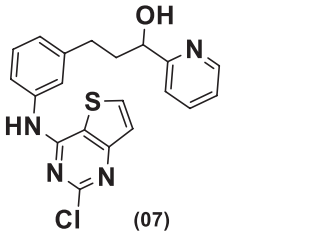
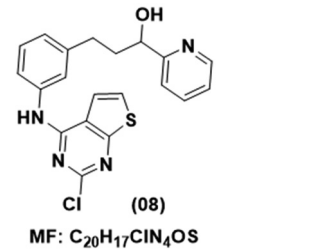
Name, appearance, molecular structure, molecular formula, molecular weight, molecular mass ion determined by mass spectrometry, and HPLC purity (area %) are presented in Table 1.

Elemental analysis and molecular mass ion determined by mass spectrometer supported the molecular formula of the synthesized pyrimidine derivatives. The synthesized pyrimidine derivatives exhibited characteristic vibration frequency in IR spectra at around 3250–3300 cm^{-1} for (OH), at 2920–2978 cm^{-1} for (C-H, aromatic), at 1620–1699 cm^{-1} for (C=O), around 1570–1596 cm^{-1} for (C=C),at 1525–1575 cm^{-1} for (C=N, aromatic), and at around 700 cm^{-1} for (C-Cl) supporting the presence of functional groups in suggested pyrimidine derivatives. Important vibration frequencies in pyrimidine derivatives are reported in Table 2.

^{1}HNMR spectra were recorded in Bruker Avance, DPX 300. Peaks for aromatic proton were observed in between 9.16 δ and 6.5 δ. Peaks for aliphatic protons are coming in between 1.6 δ and 4.6 δ. Number of peaks, relative intensities, and fine structures comply with the proposed structures of pyrimidine derivatives. ^{1}HNMR data of pyrimidine derivatives are presented in Table 2.

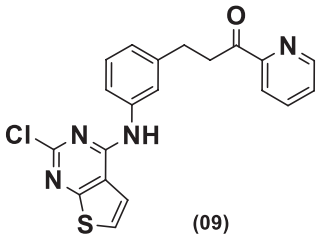
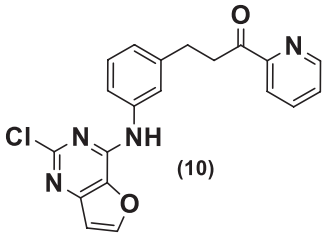
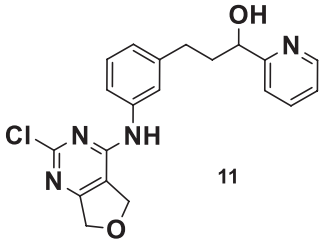
The synthesized pyrimidine derivatives have been screened for antibacterial activity against the bacteria *Escherichia coli* and *Bacillus sphaericus*. Antifungal activity of pyrimidine derivative has been screened against fungus *Aspergillus niger* and *Penicillium funiculosum*. Significant antibacterial activity was observed in case of compounds 10 and 11, moderate-to-good antibacterial activity was observed in case of compounds 5, 6, and 8. Compounds 5, 6, and 10 exhibited significant antifungal activity against *P. funiculosum*, whereas moderate antifungal activity was observed in case of compounds 7 and 8 against *A. niger*. Antibacterial

Table 2: IR, NMR spectral data of pyrimidine derivatives

S. No.	Compound	IR frequency (in/cm)	^{1}H NMR (in DMSO-d6) chemical shift in ppm
1	 (05) MF: $C_{20}H_{15}ClN_4OS$ MW: 394.88	1625 cm^{-1} (C=O), 1571 cm^{-1} (C=C, aromatic), 1526 cm^{-1} (C=N), 697 cm^{-1} (C-Cl)	10.03 (s, 1H), 9.16 (dd, *J*=2.3, 0.9 Hz, 1H), 8.79 (dd, *J*=4.8, 1.7 Hz, 1H), 8.33 (dt, *J*=8.0, 2.0 Hz, 1H), 7.85 (d, *J*=6.0 Hz, 1H), 7.79–7.52 (m, 4H), 7.51–7.25 (m, 1H), 7.12 (d, *J*=7.6 Hz, 1H), 3.49 (t, *J*=7.4 Hz, 2H), 3.00 (t, *J*=7.4 Hz, 2H)
2	 (06) MF: $C_{20}H_{15}ClN_4O_2$ MW: 378.82	1634 cm^{-1} (C=O), 1596 cm^{-1} (C=C, aromatic), 1566 cm^{-1} (C=N), 698 cm^{-1} (C-Cl)	δ 10.32 (s, 1H), 9.16 (d, *J*=2.2 Hz, 1H), 9.01–8.67 (m, 2H), 8.53–8.26 (m, 2H), 7.66–7.52 (m, 3H), 7.30 (t, *J*=7.8 Hz, 1H), 7.13–7.00 (m, 1H), 3.47 (t, *J*=7.4 Hz, 2H), 2.98 (t, *J*=7.3 Hz, 2H)
3	 (07) MF: $C_{20}H_{17}ClN_4OS$ MW: 396.89	3225 cm^{-1} (-OH), 1596 cm^{-1} (C=C, aromatic), 1530 cm^{-1} (C=N), 697 cm^{-1} (C-Cl)	δ 10.12 (s, 1H), 8.48 (ddd, *J*=4.8, 1.8, 1.0 Hz, 1H), 8.26 (d, *J*=5.4 Hz, 1H), 7.79 (td, *J*=7.7, 1.9 Hz, 1H), 7.62–7.19 (m, 6H), 7.05 (d, *J*=7.5 Hz, 1H), 5.50 (d, *J*=5.1 Hz, 1H), 4.62 (dt, *J*=8.9, 4.6 Hz, 1H), 2.70 (t, *J*=7.9 Hz, 2H), 2.14–1.77 (m, 2H)
4	 (08) MF: $C_{20}H_{17}ClN_4OS$ MW: 396.89	3250 cm^{-1} (-OH), 1572 cm^{-1} (C=C, aromatic), 1530 cm^{-1} (C=N), 696 cm^{-1} (C-Cl)	δ 9.99 (s, 1H), 8.48 (d, *J*=3.5 Hz, 1H), 7.96–7.62 (m, 3H), 7.53 (d, *J*=9.0 Hz, 2H), 7.42–7.20 (m, 3H), 7.02 (d, *J*=7.6 Hz, 1H), 5.50 (d, *J*=5.1 Hz, 1H), 4.61 (s, 1H), 2.70 (t, *J*=8.1 Hz, 2H), 1.93 (d, *J*=7.8 Hz, 2H)

(Contd...)

Table 2: *(Continued)*

5	 (09) MF: $C_{20}H_{15}ClN_4OS$ MW: 394.88	1692 cm^{-1} (C=O), 1573 cm^{-1} (C=C, aromatic), 1526 cm^{-1} (C=N), 697 cm^{-1} (C-Cl)	10.02 (s, 1H), 8.73 (d, *J*=4.9 Hz, 1H), 8.00 (d, *J*=7.0 Hz, 2H), 7.85 (d, *J*=6.0 Hz, 1H), 7.78–7.62 (m, 3H), 7.58 (s, 1H), 7.33 (t, *J*=7.8 Hz, 1H), 7.08 (d, *J*=7.7 Hz, 1H), 3.57 (t, *J*=7.5 Hz, 2H), 3.00 (t, *J*=7.5 Hz, 2H)
6	 (10) MF: $C_{20}H_{15}ClN_4O_2$ MW: 378.82	1699 (C=O), 1591 cm^{-1} (C=C, aromatic), 1567 cm^{-1} (C=N), 697 cm^{-1} (C-Cl)	10.32 (s, 1H), 8.84–8.56 (m, 1H), 8.40 (d, *J*=2.2 Hz, 1H), 8.09–7.91 (m, 2H), 7.77–7.53 (m, 3H), 7.35–7.14 (m, 1H), 7.14–6.82 (m, 2H), 3.56 (t, *J*=7.5 Hz, 2H), 2.98 (t, *J*=7.5 Hz, 2H)
7	 11 MF: $C_{20}H_{19}ClN_4O_2$ MW: 382.85	3309 (-OH), 1570 cm^{-1} (C=C, aromatic), 1526 cm^{-1} (C=N), 696 cm^{-1} (C-Cl)	9.54 (s, 1H), 8.49 (dd, *J*=4.9, 1.8 Hz, 1H), 7.92–7.79 (m, 1H), 7.54 (dd, *J*=8.4, 5.1 Hz, 2H), 7.43–7.25 (m, 2H), 6.98 (d, *J*=7.8 Hz, 1H), 6.55–6.43 (m, 1H), 5.53 (s, 1H), 4.92 (s, 2H), 4.83 (t, *J*=2.7 Hz, 2H), 4.61 (dt, *J*=8.1, 4.0 Hz, 1H), 2.68 (t, *J*=7.8 Hz, 2H), 2.15–1.80 (m, 2H)

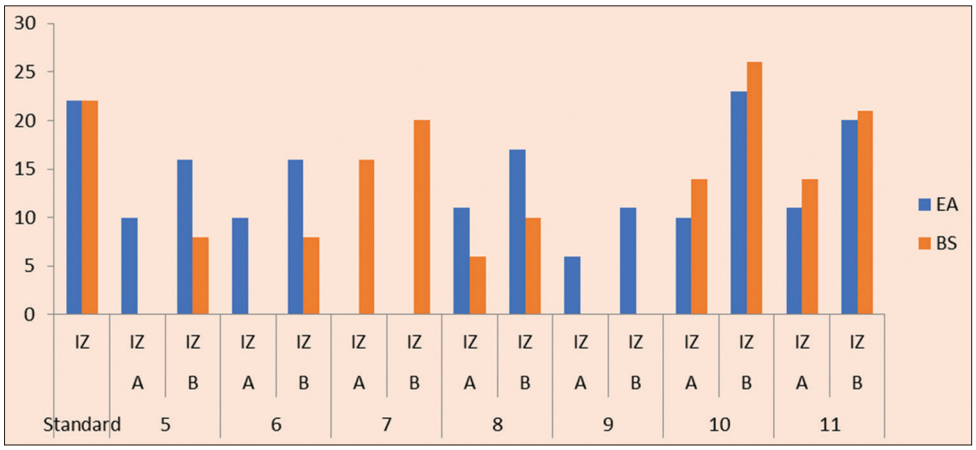


Fig 1: Graphical representation of antimicrobial activity of synthesis compound I.Z.=Inhibition zone showed by extract against microorganism in mm; EA=*Escherichia coli* (MTCC- 1559), BS=*Bacillus sphaericus* (MTCC-7542), inhibition zone; A=40 µl/ml; B=80 µl/ml; Ciprofloxacin standard; 22(mm)

activity of pyrimidine derivatives is presented in Table 3 and Fig 1, whereas antifungal activity of pyrimidine derivatives is presented in Table 4 and Fig 2.

CONCLUSION

Novel pyrimidine derivatives (compounds 5–11) were synthesized and screened against *E. coli* and *B. sphaericus* with reference to Ciprofloxacin and against fungal strains *A. niger* and *P. funiculosum* with reference to Ketoconazole at the concentration of 1 µg/mL. Good-to-moderate antibacterial and antifungal activities were observed in some of the newly synthesized pyrimidine derivatives.

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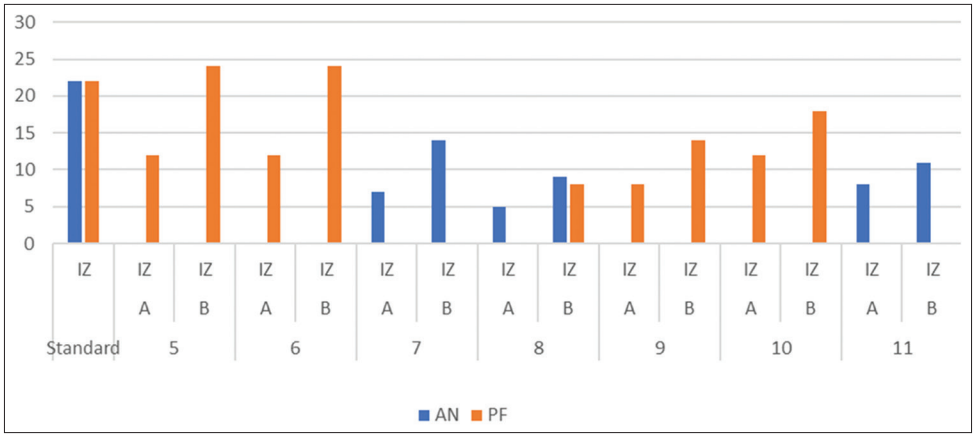

Fig 2: Graphical representation of antimicrobial activity of synthesis compound. I.Z.=Inhibition zone showed by extract against microorganism in mm; EA=*Penicillium funiculosum* (MTCC-3772), AN=*Aspergillus niger* (MTCC-9652), inhibition zone; A=40 µl/ml; B=80 µl/ml; Ketoconazole standard: 22 (mm)

Table 3: Antibacterial activity of pyrimidine derivatives

ID of compound	Concentration	Inhibition zone in mm	
		EA	BS
Standard		22	22
5	A	10	0
	B	16	8
6	A	10	0
	B	16	8
7	A	0	16
	B	0	20
8	A	11	6
	B	17	10
9	A	6	0
	B	11	0
10	A	10	14
	B	23	26
11	A	11	14
	B	20	21

Table 4: Antibacterial activity of pyrimidine derivatives

ID of compound	Concentration	Inhibition zone in mm	
		AN	PF
Standard		22	22
5	A (40 µl/ml)	0	12
	B (80 µl/ml)	0	24
6	A (40 µl/ml)	0	12
	B (80 µl/ml)	0	24
7	A (40 µl/ml)	7	0
	B (80 µl/ml)	14	0
8	A (40 µl/ml)	5	0
	B (80 µl/ml)	9	8
9	A (40 µl/ml)	0	8
	B (80 µl/ml)	0	14
10	A (40 µl/ml)	0	12
	B (80 µl/ml)	0	18
11	A (40 µl/ml)	8	0
	B (80 µl/ml)	11	0

AUTHORS' CONTRIBUTIONS

Mr. Bhagchand Jat and Dr. Swapna Santra contributed to design and to develop main conceptual ideas. Bhagchand Jat performed the experiment and compiled the analytical data, drafted the manuscript. Dr. Swapna Santra and Dr. Prasanta Kumar Santra interpreted the results and reviewed the manuscript. All authors discussed the results and approved final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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