International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF PYRIMIDINE DERIVATIVES

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ABSTRACT

A series of 3-amino-4,6-diphenyl-3,4-dihydro-1H-pyrimidine-2-thione derivatives have been synthesized by cyclization of substituted chalcones with thiosemicarbazide. The structures of all newly synthesized compounds were confirmed by FT- IR, ^{1}H NMR and Mass spectral analysis. The synthesized compounds were screened for antibacterial activity (500µg/ml) concentration level by disc diffusion method against (Gram negative) *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia*.

Keywords: Chalcone, Pyrimidine, Thiosemicarbazide hydrochloride.

INTRODUCTION

Heterocyclic compounds are abundant in nature and are of great significance to life because their structural subunits exist in many natural products such as vitamins, hormones, antibiotics etc. A practical method for the synthesis of such compounds is of great interest in synthetic organic chemistry [1]. Among a wide variety of heterocycles that have been explored for developing medicinally important molecules, pyrimidine derivatives occupy an important place in the present day therapeutics[2].
Pyrimidines are six membered heterocyclic ring compounds composed of nitrogen and carbon. It can be regarded as a cyclic amine and also be known as m-diazine (or) 1,3-diazine [3]. Pyrimidine and their derivatives are considered to be important for drugs and agricultural chemicals. As pyrimidine is a basic nucleus in DNA & RNA, it has been found to be associated with diverse biological activities[4]. Compound containing pyrimidine ring systemcan be found in the nucleoside antibiotics, antibacterial[5], antitumor[6], anticancer [7],cardiovascular, antifolateas well as agrochemical and veterinary products[8]. Pyrimidine frequently used in medicine such as flucytosine, Idoxuridine[9] or pyrimidine ring in fused form with other heterocycles (methotrexate, prazocin) [10] and in some drugs pyrimidines are commonly employed as substituents or lead moieties. Bacterial and fungal diseases are the most common all over the world. Though, many antibiotics are currently marketed, they have a tendency of becoming resistant and are prone to severe adverse effects after long term use. Hence, there is a never lasting demand in synthesis of novel antimicrobial agents having good potency, efficacy with lesser side effects[11]. The current article is aimed at synthesis of halogenated pyrimidine analogs that are active against common pathogenic bacteria. In view of newer antibacterial is focused on synthesis and evaluation of pyrimidine derivatives.

MATERIAL AND METHODS

All the reagents and solvents used were of analytical grade .The melting points were determined by open tube capillary method. The synthesized compounds characterized by FT-IR, ^{1}H NMR and mass spectroscopy. IR spectra were recorded using Perkin Elmer FT-IR(89258) spectrophotometer using KBr discs. A ^{1}H NMR spectrum was recorded using DMSO on Bruker Avance II400 spectrometer and their chemical shifts were recorded in δ (parts per million) units with respect to tetramethylsilane

(TMS) as internal standard. Mass spectra were recorded on a Waters Q-T of micro MS.

Synthesis and characterization of pyrimidine derivatives:

Step 1. Synthesis of chalcones derivatives

Chalcones were synthesized by base catalyzed Claisen-Schmidt condensation reaction of appropriately substituted acetophenone and aldehydes by known literature method[12].

A mixture of Acetophenone derivatives(0.1mol) andBenzaldehyde derivatives(0.1mol) were taken in reaction vessel and dissolved in 30ml ethanol. To this solution 40% sodium hydroxide was added. Then the reaction mixture was subjected for microwave irradiation for duration of 4 minutes at 160 watts.The reaction mixture was neutralized with concentrated hydrochloric acid, and then the solid separated was collected and crystallized from ethanol to get the chalcone derivatives.

Step 2. Synthesis of 3-amino-4,6-diphenyl-3,4-dihydro-1H-pyrimidine-2-thione derivatives.

A mixture of chalcone derivative (0.1 mol) and thiosemicarbazide hydrochloride (0.1 mol) and alcoholic solution of sodium hydroxide were taken into a reaction flask and heated under reflux for 4-5 hrs. The excess of solvent was removed by distillation. The concentrate was then diluted with cold water and cooled further. The solid mass thus resulted was filtered, washed with small portion of cold water and dried. It was purified by recrystallization from alcohol to get yellow crystalline solid and analogues of pyrimidine were prepared by same method with good yield[13].The synthetic route of synthesized compound shown in**Fig No. 1.**

Antibacterial activity

The antibacterial activity of all synthesized compounds were determined by disc diffusion Method[14]. All human pathogenic bacteria viz*E.coli*, *Pseudomonas aeruginosa, Klebsiella pneumonia* were procured from SGRRITS, Dehradun. The stock cultures were obtained from stock culture bank of microbiology laboratory of SGRRITS, Dehradun and maintained on nutrient agar media slants at 4°C. In order to activate these cultures, subculture, were freshly prepared and incubated at 37°C for 18 hrs to 24 hrs before use. The nutrient agar medium and peptone diameter were punched from whatman No.1 filter paper. Stock solutions of synthesized compounds diluted in dimethyl sulphoxide (1% DMSO) to give final concentration of 500µg/ml. A reference standard for gram negative bacteria wasmade by dissolving accurately weighed quantity of erythromycin (500µg/mL respectively) in sterile distilled water. The incubation was carried out at 37ºC for 24h. All the experiments were carried out in triplicate. Simultaneously, controls were maintained by employing 0.1 mL of dimethylsulfoxide which didnot reveal any inhibition. The clear zone ofinhibition around paper dics demonstrated the relative susceptibility towards the synthesized derivatives. Zones of inhibitionproduced by each compound was measured in mm.

RESULTS AND DISCUSSIONS

In this study we have prepared new 3-Amino-4,6-diphenyl-3,4-dihydro-1H-pyrimidine-2-thione derivatives. The initial step in the synthetic method involved the synthesis of five different chalcones from different substituted acetophenones and benzaldehyde, followed by cyclization with thiosemicarbazide. The physical constants were shown in **Table No.1**.

The synthesized compounds characterized by FT-IR, ^{1}HNMR and Mass spectroscopy. The titled compounds were confirmed by IR spectral data showing the presence of N-H bands in theregion of 3000 -3300 cm $^{-1}$, (C=C) bands at 1600-1475 cm $^{-1}$ and (C-H) bands at 2850-3000 cm $^{-1}$[15]. In the ^{1}H NMR spectra, all protons were seen according to the expected chemical shift and integral values[16]. Mass spectra of the compounds showed molecular ion peaks with high abundance at m/z in agreement with their molecular formula[17] .The spectral data of compounds shown in **Table No. 2**.

All the five synthesized compounds were studied for their antibacterial activity.The results revealed that majorityof the synthesized compounds showed varying degrees ofinhibition against Gram negative bacteria shown in **Table No. 3**. The compound AY4(3-Amino-4-(4-chloro-phenyl)-6-phenyl-3,4-dihydro-1H-pyrimidine-2-thione) has shown maximum activity against gram negative bacteria at 500µ/ml concentration level and the compound AY2 (3-Amino-4-(4-chloro-phenyl)-6-(4-methoxy-phenyl)-3,4 dihydro-1H-pyrimidine-2-thione) has shown moderate activity against gram negative bacteria. The compound AY5 (3-Amino-6-(4-bromo-phenyl)-4-phenyl-3,4-dihydro-1H-pyrimidine-2-thione)showed least activity against *Pseudomonas aeruginosa* whereas compound AY5 showed no activity against *E.coli* and *Klebsiella Pneumonia* at concentration 500µg/ml.The results of the present investigation encourage us to develop more moieties and test them for wide range of biological activities.

CONCLUSION

A new series of 4-phenyl substituted pyrimidine-2(1H)-thiones were synthesized. The synthesized compound were characterized by FT-IR,[1]HNMR, Mass spectroscopy and evaluated for Antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia*. The screening result revealed that the compound (AY1– AY5) showed significant antibacterial activity at 500µg/ml concentration level when compare with standard drug (Erythromycin).

ACKNOWLEDGEMENT

Authors owe a deep sense of gratitude to his holiness Shri DevendraDassJiMaharaj, for providing facilities for this work.

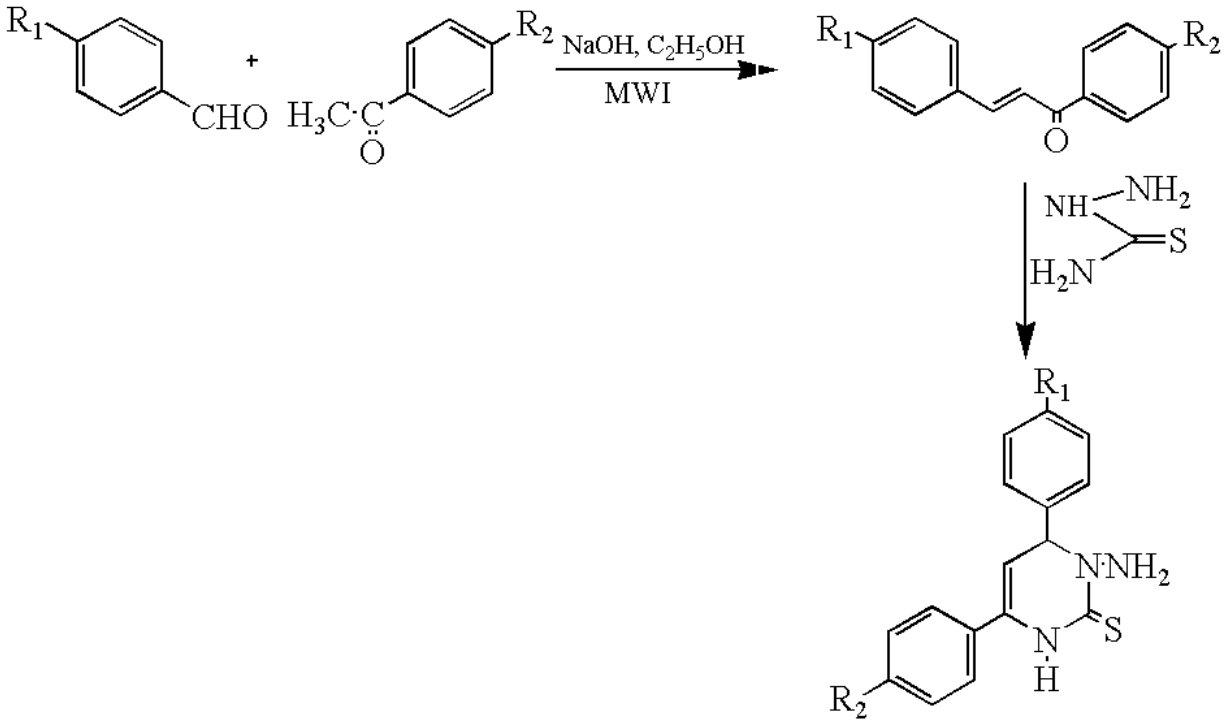


Fig 1. Synthetic route to the title compounds

Table No. 1. Physical constants of the synthesized compounds.

Compound Code	R1	R2	Mol. Formula	Melting Point	% Yield	Mol. Wt
AY1	H	H	$C_{16}H_{15}N_3S$	172-174	76%	282.1
AY2	Cl	OCH3	$C_{17}H_{16}ClNOS$	231-233	58%	346.0
AY3	Cl	Br	$C_{16}H_{13}BrClN_3S$	255-257	79%	396.0
AY4	Cl	H	$C_{16}H_{14}ClN_3S$	212-214	64%	316.1
AY5	H	Br	$C_{16}H_{14}BrN_3S$	192-195	72%	360.0

Table No.2. The spectral data of synthesized compounds:

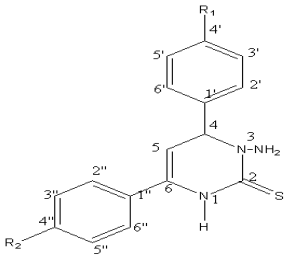
Compound code	IR (cm_{-1})	Mass (ESI)	^{1}HNMR (δ in CDCl3), ppm
			
AY1	3483.87 (str, NH_2), 3349.8 (str, NH), 2921.8, 2851.22 (str, C-H), 1069.94 (str, C=S), 1574.34 (str, C=C)	m/z(relative intensity%): 282.1$[M+1]^+$ 304.0$[M+Na]^+$	7.89-7.92 (d, J=12Hz, 2H, Ar 3',5'H), 7.82(m, 1H Ar 4''H), 7.76-7.78 (d,J= 8Hz, 2H, Ar 3'',5'' H), 7.40(m, 1H,Ar 4' H),7.18-7.21 (d, J=12Hz, 2H, Ar 3'5'H),7.14-7.16 (d, J=8Hz, 2H, Ar 2'6'H), 5.94-5.98 (m, J=16Hz, =CH), 3.81-3.86 (m, J= 20 Hz, -CH), 2.54 (s, 1H, NH_2), 2.33 (d, 2H, -NH)
AY2	616.47 (str, C-Cl), 3437.78 (str, NH_2), 1298.05 (str, C-O), 3352.54 (str, N-H), 1086.35 (str, C=S), 2922.06, 2851.05 (str, C-H)	m/z(relative intensity%): 346.0 $[M+1]^+$ 368.0$[M+Na]^+$	7.33-7.37 (d, J=16Hz,2H, Ar 3'',5''H), 7.25-7.27 (d, J=8Hz, 2H, Ar 3'5'H), 7.11-7.13(d, J=8Hz,2H, Ar 2'',6''H),6.90-6.92 (d, J=8Hz, 2H, Ar 2',6'H), 5.90-5.91 (m, J=4Hz, =CH), 3.29-3.32 (m, 3H, 4''), 2.25 (d, 1H, -NH), 1.21 (s, 2H, NH_2)
AY3	759.34 (str, C-Cl), 661.73 (str, C-Br), 3476.15 (str, NH_2), 3353.46 (str, N-H), 2921.34, 2851.04(str, C-H),	m/z(relative intensity%): 394.0$[M+]^+$ 396.0$[M+2]^+$	7.29-7.31(d, 2H,J= 8Hz, Ar 3'',5''H),7.16-7.21(d, 2H, J=20Hz, Ar 2'',6''H), 7.07-7.12(d, 2H, J=20Hz, Ar 3',5'H), 6.92-6.94 (d, 2H, J=8Hz, Ar 2',6' H), 5.71-5.72 (m, J= 4Hz, =CH), 3.62-3.66 (m, J=16Hz, -CH), 2.28(d, 1H,-NH), 2.07 (s, 2H ,-NH_2)
AY4	758.94 (str, C-Cl), 3437.34 (str, NH_2), 3277 (str, N-H), 2850.90, 2920.98(str, C-H), 1525.2(str, C=C)	m/z(relative intensity%): 316.1$[M+1]^+$	7.76-7.81 (dd, 2H, J=20Hz, Ar 2'',6''),7.36-7.40 (dd, 2H, J=16Hz, Ar 3'',5''H), 7.27-7.28 (d, 2H , J= 4Hz, Ar 3'5'H), 7.13-7.14 (d, 2H, J=4Hz, Ar 2'6'H), 5.91-5.95 (m, J=16Hz, =CH), 3.83-3.88 (m, J= 20Hz, -CH), 2.53 (s, 2H, NH_2), 2.30 (d, 1H, -NH)
AY5	553.44(str, C-Br), 3483.22 (str, NH_2), 3349.28 (str, NH), 2921.4 (str, C-H), 1071.53 (str, C=S)	m/z(relative intensity%): 360.0$[M+]^+$ 362.0[M+2]	7.77-7.79 (d, 2H,J=8 Hz, Ar 3'',5''H),7.57-7.59 (d, 2H, J=8Hz, Ar 2'',6''H), 7.49-7.51 (d, 2H, J=8Hz, Ar 3',5'H), 7.28-7.30 (m, 1H, 4'), 7.12-7.14 (d, 2H,J=8Hz, Ar 2',6'H),5.92-5.93 (m, J= 4Hz, =CH), 3.83-3.87 (m, J=16Hz, -CH), 2.49 (d, 1H, -NH), 2.28 (s, 2H, NH_2)

Table No. 3. Zone of inhibition obtained on bacteria:

Compound Code	***E.coli***		***Klebsiella pneumonia***		***Pseudomonas aeruginosa***	
	500µg/ml	% Inhib.	500µg/ml	% Inhib.	500µg/ml	% Inhib.
AY1	2.2	11.22%	1.8	11.11%	2.8	14.5%
AY2	5.8	29.5%	7.1	43.8%	7.5	39.0%
AY3	2.8	14.3%	2.7	16.6%	3.4	17.7%
AY4	15.0	76.5%	9.8	60.4%	14.8	77.0%
AY5	-	-	-	-	1.0	5.20%
Erythromycin	19.6	-	16.2	-	19.2	-
DMSO	-	-	-	-	-	-

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