SYNTHESIS OF 4-[4-(4-ETHYL-PIPERAZIN-1-YL)-PHENYL]-6-PHENYL-3,4-DIHYDRO-1H-PYRIMIDINE-2-THIONE (THIOPYRIMIDINE) DERIVATIVES FROM 3-[4-(4-ETHYL-PIPERAZIN-1-YL)-PHENYL]-1-PHENYL-PROPENONE (CHALCONES) DERIVATIVES AND THEIR ANTIMICROBIAL STUDIES

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ABSTRACT

A series of new substituted of 4-[4-(4-Ethyl-piperazin-1-yl)-phenyl]-6-phenyl-3,4-dihydro-1H-pyrimidine-2-thione (thiopyrimidine) derivatives were synthesized from substituted 3-[4-(4-Ethyl-piperazin-1-yl)-phenyl]-1-phenyl-propenone (chalcones) derivatives and thiourea by using NaOH as catalyst in ethanol at reflux temperature. The newly Synthesized thiopyrimidines were confirmed by TLC, melting points, IR, ^{1}H-NMR and mass spectra. The compound were evaluated for antibacterial activity against *Escherishia coli, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis* and antifungal activity against *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, Candida albican.* All the compounds shows moderate to good activity against different micro-organisms.

Keywords: 1-Ethyl-piperazine, Chalcones, thiourea , thiopyrimidnes, antimicrobial activity.

Introduction

Heterocyclic rings have played a significant role in medicinal chemistry by serving as key templates central to the development of numerous important therapeutic agents. Pyrimidine derivatives

have found a wide applications in medicinal chemistry because of their diverse biological activities as well as Pyrimidine nucleus exhibited remarkable pharmacological activities. The 4-aryl-1, 2, 3, 4-

tetrahydropyrimidines have been given the name Biginelli compounds. The main interest in Biginelli compounds however is due to the strong antihypertensive activity exhibited by certain derivatives. Also a large number of substituted pyrimidines have been documented to have several biological activities[1].

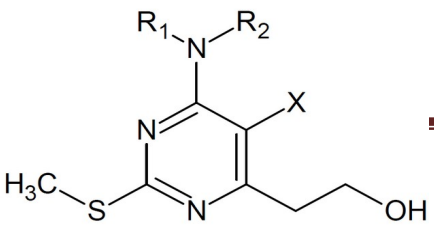
Thiopyrimidines are excellent reservoir of bioactive substances. The important

pharmacological activities known from literature are Antifilarial[2] , Antiinflammatory and analgesic[3], Antileishmanial[4], Anticancer and Herbicidal[5], Antineoplastic[6], Antiviral and Antitumor[7,8], Antimicrobial[9], AntiAIDS and Antitumor[10], Antitubercular[11], Antileishmanial and Antiviral[12] , Antagonists[13] and Herbicidal[14].

Due to interesting activity of various substituted pyrimidines as biological agents, considerable attention has been focused on this class. One of the methods for the synthesis of such compound is from α,β-unsaturated carbonyl compound by cyclization with urea or thiourea to yield the thiopyrimidine nucleus.

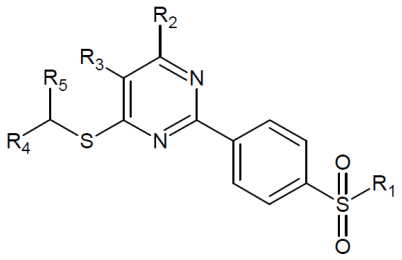
Supaluk Prachayasittikul[15] *et al* have reported synthesis of novel analogs of bioactive 2-substituted thiopyrimidines-4-(3*H*)-ones *via* base catalyzed alkylation reaction of 2-thiouracil using alkyl and aralkyl bromides and tested the bioactivity of synthesized compounds. Tests revealed that thiopyrimidines exhibited antimicrobial activity. The thiopyrimidine-4-one showed complete inhibition against *Streptococcus pyogenes* and *Branhamella catarrhalis* as well as antifungal action against *Candida albicans*. Significantly, the 1-adamantylthiopyrimidine was shown to be the most potent cytotoxic compound against multidrugresistant small cell lung cancer (H69AR).

C. Mugnaini[16] *et al* have synthesized 4-alkylamino-6-(2-hydroxyethyl)-2-methylthiopyrimidines and reported them as new rubella virus inhibitors.



Vyacheslav E. Semenov[17] *et al* have reported the antimicrobial activity of pyrimidinophanes with thiocytosine and uracil moieties. K. B. Puttaraju[18] *et al* have reported the microwave synthesis of pyrimidines and their *in vitro* antimicrobial and anticancer activities

G. Liu[19] *et al* have synthesized novel 6-alkylamino-2,4-dialkyl(aryl) thiopyrimidines, which showed antiplatelet activity. H. O. Kim[20] *et al* have synthesized novel D-2'-Azido-2',3'-dideoxyarabinofuranosyl -4'-thiopyrimidines and tested their biological activities. Aurelio Orjales[21] *et al* have reported novel 2-(4-methylsulfonylphenyl)pyrimidine derivatives as highly potent and specific COX-2 inhibitors.



L. G. Hammerland[22] *et al* have studied the structure–activity relationship of thiopyrimidines as mGluR5 antagonists. S. M. Rajesh[23] *et al* have reported a green expedient synthesis of pyridopyrimidine-2-thiones. The synthesized compounds were screened for their *in vitro* activity against *Mycobacterium tuberculosis* H37Rv. Among them, (*E*)-6-benzyl-8-(2,4-dichlorobenzylidene)-4-(2,4-dichlorophenyl)-3,4,5,6,7,8-hexahydropyrido[4,3-d]pyrimidine-2(1H)-thione (MIC 2.8 lM) displays the maximum activity, being 2.7 and 1.7 times more active than the first line antitubercular drugs Ethambutol and Ciprofloxacin, respectively.

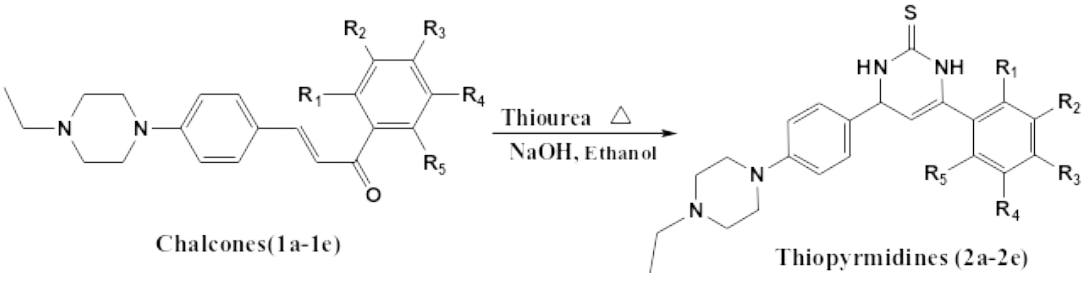
Material and Methods

Melting points were determined in open capillary tubes and are uncorrected. The purity of the compounds was checked by TLC on silica gel G. UV light or iodine vapour accomplished visualization. The IR Spectra were recorded on FTIR perkin-Elmer 1420 spectrometer and PMR spectra ($CDCl_3$) on a varian-300 MHZ spectrometer using TMS as internal standard. Mass spectra were recorded on VG 7070 H Mass spectrometer at 70 eV.

Synthesis of Thiopyrimidines Typical Procedure

A mixture of chalcone (1mmol) and thiourea (1 mmol) in presence of NaOH as catalyst in ethanol (15 mL) was refluxed for 2-3 hours. After completion of the reaction (TLC), the reaction mixture was cooled and poured into ice cold water (100 ml.). The separated solid was filtered, washed with ice cold water and recrystallized from ethanol. The purity of synthesized thiopyrimidine was checked by TLC

synthesis of substituted 4-[4-(4-Ethyl-piperazin-1-yl)-phenyl]-6-phenyl-3,4-dihydro-1H-pyrimidine-2-thione (2a-2e) were carried out starting from substituted 3-[4-(4-Ethyl-piperazin-1-yl)-phenyl]-1-phenyl-propenone (1a-1e) derivatives by using thiourea in NaOH as catalyst in ethanol at reflux temperature..



Chalcones(1a-1e)

Thiopyrmidines (2a-2e)

Entry	Product	R_1	R_2	R_3	R_4	R_5
1	2a	H	H	H	H	H

2	2b	OH	H	H	H	H
3	2c	H	H	Br	H	H
4	2d	H	H	F	H	H
5	2e	H	H	CH_3	H	H

Procedure for synthesis of 4-[4-(4-Ethyl-piperazin-1-yl)-phenyl]-6-phenyl-3,4-dihydro-1H-pyrimidine-2-thione (2a):

A mixture of 3-[4-(4-Ethyl-piperazin-1-yl)-phenyl]-1-phenyl-propenone (1a) (1 mmol) and thiourea (1 mmol) in presence of NaOH as catalyst in ethanol (15mL) was refluxed for 2 hours. After completion of the reaction (TLC), the reaction mixture was cooled and poured into ice cold water (100 ml). The separated solid was filtered, washed with ice cold water and recrystallized from ethanol.

Similarly, remaining compounds of this series were also prepared by same procedure. The physical data of synthesized compounds are tabulated as in the table 1

RESULTS AND DISCUSSIONS

The Thiopyrimidines formation is the reaction between chalcone and thiourea. Here we have used the substituted 3-[4-(4-Ethyl-piperazin-1-yl)-phenyl]-1-phenyl-propenone (1a-1e) derivatives

The reaction is carried out in ethanol solvent in presence of NaOH base as a catalyst. Reaction took 2 to 3 hours for completion and give a good yield of thiopyrimidines.

Table 1: The physical data of synthesized thiopyrimidine derivative (2a-2e).

Entry	Product	Mol. Formula	Yield %	M.P.(°C)
1	2a	$C_{22}H_{26}N_4S$	76	265
2	2b	$C_{22}H_{26}N_4OS$	84	186
3	2c	$C_{22}H_{25}BrN_4S$	78	167
4	2d	$C_{22}H_{25}FN_4S$	75	245
5	2e	$C_{23}H_{28}N_4S$	87	174

The structure of the synthesized compounds was confirmed by IR, ^{1}H NMR and Mass. All the compounds give the characteristic IR peaks that proved that the presence of particular functional group, ^{1}H NMR helps to find out the number of Hydrogen atom and their environment and mass spectroscopy helps to find the molecular weight of the synthesized compounds.

Spectroscopic data of all the synthesized compounds is mentioned below

I) 4-[4-(4-Ethyl-piperazin-1-yl)-phenyl]-6-phenyl-3,4-dihydro-1H-pyrimidine-2-thione (2a):

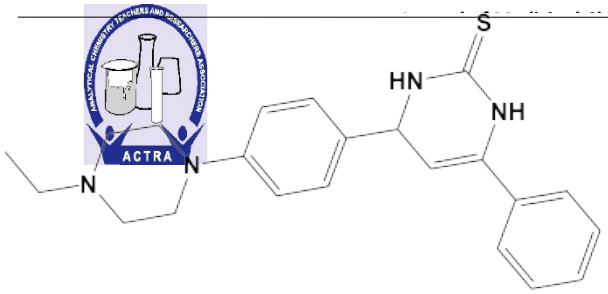
M.F.: $C_{22}H_{26}N_4S$

IR (KBr): 3189, 2967, 2820, 1608, 1516, 1451, 1235, 1182, 950, 873, 822, 763, 696, cm^{-1}.

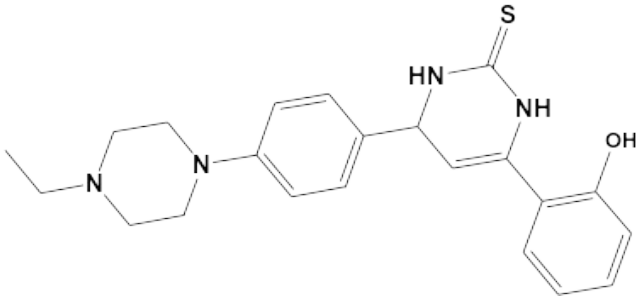
^{1}HNMR: δ 1.0 (t, 3H, CH_3), δ 2.3 (q, 2H, CH_2), δ 2.45 (t, 4H, CH_2), δ 3.35 (t, 4H, CH_2),

δ 5.0 (s, 1H, NH), δ 5.35 (s, 1H, NH), δ 6.65-8.3 (m, 10H, Ar-H & 5H Thiopyrimidine), δ 8.8 (s, 1H, 6H of Thiopyrimidine).

M.S. (m/z): 378(M+).



II) 4-[4-(4-Ethyl-piperazin-1-yl)-phenyl]-6-(2-hydroxy-phenyl)-3,4-dihydro-1H-pyrimidine-2-thione (2b):



M.F.: $C_{22}H_{26}N_4OS$

IR (KBr): 3777, 3191, 2939, 2854, 1627, 1543, 1512, 1485, 1342, 1276, 1188, 1122, 856, 813, 767, 696 cm^{-1}.

^{1}HNMR: δ 1.0 (t, 3H, CH_3), δ 2.3 (q, 2H, CH_2), δ 2.45 (t, 4H, CH_2), δ 3.35 (t, 4H, CH_2),

δ 5.0 (s, 1H, NH), δ 5.3 (s, 1H, NH), δ 6.8-7.7 (m, 9H, Ar-H & 5H Thiopyrimidine), δ 8.4 (s, 1H, 6H of Thiopyrimidine) δ13.1 (s, 1H, OH).

M.S. (m/z): 394 (M+)

Biological Screening For establishment of antimicrobial activity of the synthesized compounds we utilized the reported cup plate method.[24-25] The experiment is performed at a concentration of 100µg/ml. we checked the activity of these molecules against different strains of bacteria and fungi as mentioned in

table 2. DMSO was used as solvent control. The obtained data of activity of all these tested compounds is shown in **table 2**.

Table 2. Antimicrobial activity of synthesized Thiopyrimidine (2a-2e):

Product	Bacteria				Fungi			
	Ec	**St**	**Sa**	**Bs**	**An**	**Pc**	**Af**	**Ca**
2a	12	11	--	12	15	15	14	14
2b	--	15	18	15	11	15	16	15
2c	15	19	14	--	14	--	12	--
2d	12	12	15	15	11	14	15	14
2e	15	--	14	--	--	--	--	15
Penicillin	22	22	24	24	NA	NA	NA	NA
Nystatin	NA	NA	NA	NA	20	22	24	24

Zone of inhibition is expressed in mm.

An-*Aspergillus niger,*

Ec- *Escherichia coli,*

St-*Salmonella typhi,*

Pc-*Penicillium chrysogenum,*

Sa- *Staphylococcus aureus,*

Af-*Aspergillus flavus,*

Bs- *Bacillus subtillis,*

Ca-*Candida albicans,*

-- No activity, NA- Not Applicable

CONCLUSION

In conclusion, we put forth here some new thiopyrimidine using chalcones and thiourea. The reaction was clean and the products were obtained in excellent yields without formation of any side products. The synthesized compounds were characterized by TLC, melting point, IR, ^{1}H NMR and Mass spectroscopy. The results obtained from this study confirmed that the product has formed.

The compounds were evaluated for antibacterial activity against *Escherishia coli, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis* and antifungal activity against *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, Candida albican.* All the compounds shows moderate to good activity against different micro-organisms.

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