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Synthesis and antibacterial and anti-inflammatory activity of 4-substituted-thieno[2,3-*d*]pyrimidines

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ABSTRACT

*During the last few years, condensed thienopyrimidine derivatives have received considerable attention. The therapeutic importance of thienopyrimidines prompted us to synthesize certain fused thienopyrimidine derivatives with the hope of obtaining new compounds with antimicrobial and anti-inflammatory activity. Herewith it has been reported the synthesis of some novel 4-substituted thieno[2,3-d]pyrimidines as possible antibacterial and anti-inflammatory agents. Compounds **6c** and **8a** have shown good anti-inflammatory activity. The compounds **6c** and **6e** have shown moderate antibacterial activity compared to Ampicillin against Bacillus subtillis and Staphylococcus aureus.*

Keywords: Gewald reaction, 4-chlorothienopyrimidine, 4-substituted-thieno[2,3-d]pyrimidines, antibacterial, *in-vitro* protein denaturation activity.

INTRODUCTION

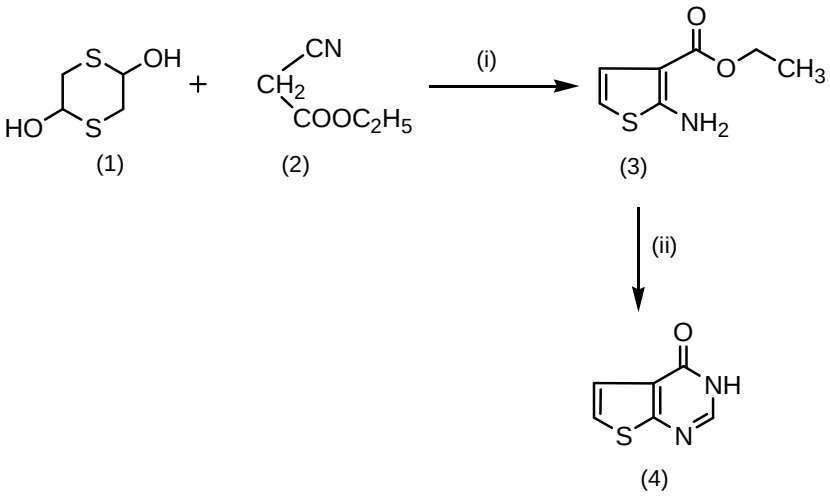
During the last few years, condensed thienopyrimidine derivatives have received considerable attention. Structural variations on the thienopyrimidine nucleus have resulted in a large number of compounds having diverse pharmacological activities. Some of the activities possessed by thienopyrimidines includes anticancer[1,2] anti-HIV[3], antiviral [4], antimicrobial [5,6], analgesic and anti-inflammatory[7], antibacterial [8], ulcerogenic[9], anticonvulsant [10]. The therapeutic importance of thienopyrimidines prompted us to synthesize certain fused thienopyrimidine derivatives with the hope of obtaining new compounds with antimicrobial and anti-inflammatory activity. Herewith we are reporting the synthesis of some novel 4-substituted thieno[2,3-*d*]pyrimidines as possible antibacterial and anti-inflammatory agents.

MATERIALS AND METHODS

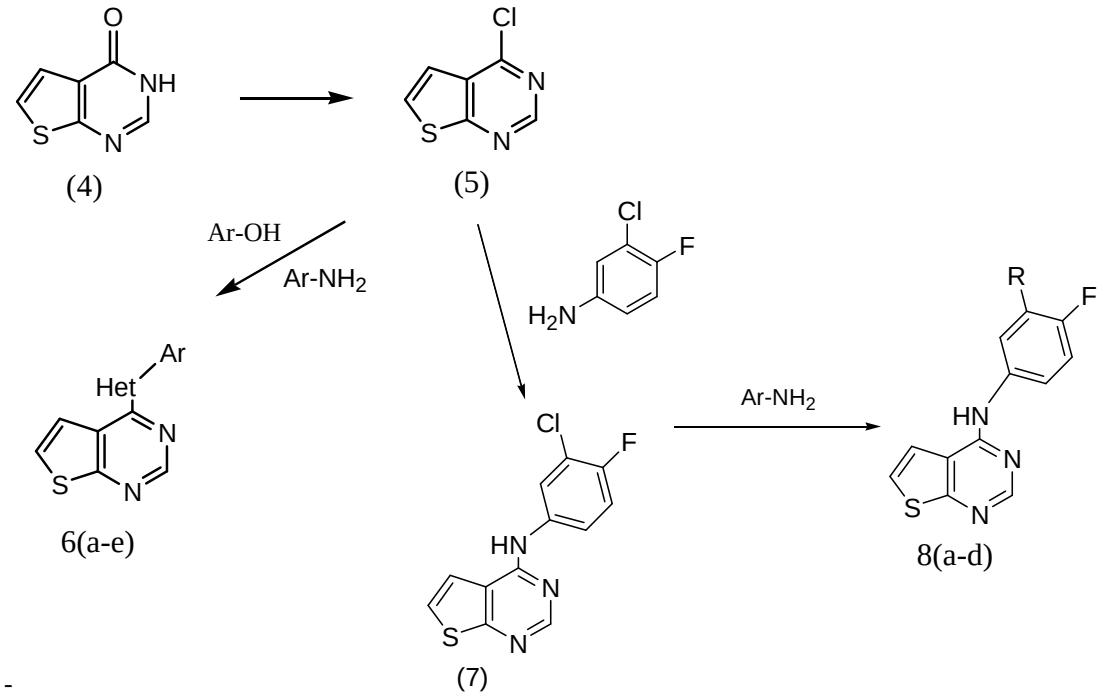
Experimental

The melting points were determined and are uncorrected. Infrared spectra (KBr disc) were performed on FTIR-8300 Shimadzu and the frequencies were expressed in cm^{-1}. ^1H NMR spectra were recorded on Bruker-Avance 400 MHz instrument with TMS (0 ppm) as an internal standard. Completion of the reaction and the purity of the compounds were checked on Merck precoated silica gel 60 F-254. Yields were not optimized. Bovine serum albumin (Merck

Limited), Ibuprofen and other chemicals were of analytical grade. All the solvents and reagents were used without further purification.



Scheme 1. (i) DMF, Triethylamine (ii) $HCONH_2$ (iii) $ClCH_2COOC_2H_5$, CH_3ONa Dry acetone



scheme-2. Syntheses of 4-substituted thienopyrimidines.

3H-Thieno[2,3-d]pyrimidin-4-one **(4)** has been synthesized by reported methods [11].

Synthesis of 4-chlorothieno[2,3-*d*]**pyrimidine (5).**

A mixture of thieno[2,3-*d*]pyrimidin-4-one (5mmol, 1gm) and Phosphorus oxychloride ($POCl_3$) (10ml) was refluxed under reduced pressure for 15 hours. The excess $POCl_3$ was removed by distillation under reduced pressure. The residue was treated with dry benzene (5ml) and the solvent distilled under reduced pressure to remove the last traces

of $POCl_3$. The residue left was triturated with ice and $NaHCO_3$ solution (10%). The solid thus obtained was collected, washed with water, dried and recrystallized from toluene.

Synthesis of 4-substituted-thieno[2,3-*d*]**pyrimidines 6(a-e).**
A mixture of 4-Chlorothieno[2,3-*d*]pyrimidine, substituted phenols, or amines and ammonium carbonate was warmed on steam bath, added in 10% solution of NaOH, heated, filtered, dried and recrystallized from chloroform to get substituted phenol and amine derivatives.

Synthesis of 4-substituted-thieno[2,3-*d*]**pyrimidines (7).**
A mixture of 4-Chlorothieno[2,3-*d*]pyrimidine, 4-Fluoro-3-Chloroaniline, ethanol, concentrated HCl was refluxed for 4 hours, cooled at room temperature. The solid was filtered, dried and recrystallized from ethanol.

Synthesis of 4-substituted-thieno[2,3-*d*]**pyrimidines 8(a-e).**
Take equimolar quantity of the compound **7** (0.01 mol) and aromatic amines(0.01 mol) and to it 5 ml of ethanol and 0.2 ml of triethyl amine was added and refluxed for 5 hours. The reaction mixture was cooled and filtered. The filtered compounds were dried and recrystallized using ethanol.

In vitro evaluation of Antibacterial activity [12-15]
All the synthesized compounds (N_3, N_5, II, N_6 and N9) were evaluated for their Antibacterial activity against *Staphylococcus aureus* and *Bacillus subtillis* at the concentration of 50µg/ml and 100µg/ml by Agar Diffusion method using $CHCl_3$ as a solvent. After 24 hours of incubation at $37^oC \pm 1\ ^oC$, zones of inhibition were measured in mm. The activity was compared with Ampicillin at the same concentration. Among the compounds tested N_3, N_5, II, N_6 and N_9 were found to be the most potent against both the microorganisms used.

In vitro anti-inflammatory activity using bovine serum albumin denaturation [16]
The test compounds were dissolved in minimum amount of DMF and diluted with phosphate buffer (0.2M, pH 7.4). Final concentration of DMF in all solution was less than 2.5%. Test solution (1ml) containing different concentrations of drug was mixed with 1 ml of 1mM albumin solution in phosphate buffer and incubated at 27° ± 1°C in a water bath for 10 min. After cooling the turbidity was measured at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. The percentage of inhibition is calculated from the following formula.

$$\% \text{ Inhibition} = 100(1-Vt/Vc)$$

Where Vt = absorbance value in test solution.
Vc = absorbance value in control solution.

RESULTS AND DISCUSSION

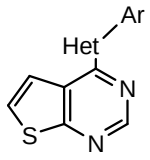
The compound **(3)** was formed by the condensation of ethylcyanoacetate with 2,5-dihydroxy-1,4-dithiane in presence of triethylamine. Compound **(4)** was prepared through condensation reaction between formamide and compound **(3)** followed by cyclisation. IR and NMR spectra confirmed the formation of compound **(4)**. The compound **(4)** as it exists in two tautomeric forms IR spectra showed the presence of ketone at 1660 cm^{-1} and NMR spectra showed a broad peak which was not prominent due to tautomerism. Absence of doublet due to NH_2 in IR spectra, absence of quartet and triplet due to $-CH_2CH_3$ at 2-4 ppm in NMR spectra confirms the cyclisation.

Chlorination has been done by using Phosphorus oxychloride according to the reported literature. Further Chlorine has been replaced by different phenols and primary amines **[6(a-e), 7]** to get the desired derivatives. In compound **7** Chlorine will be displaced by different primary amines to get **8(a-e).** The physical and spectral data of the synthesized compounds have been given in **Table 1** and **Table 2**.

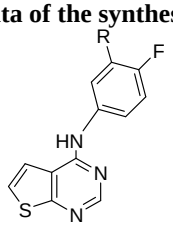
The compounds **6c, 6e, 7, 8a, 8d** were screened for antibacterial activity **(Table 3)** against various Gram positive and Gram negative bacteria using agar diffusion method. The compounds **6c** and **6e** have shown moderate antibacterial activity compared to Ampicillin against *Bacillus subtillis* and *Staphylococcus aureus*.

The compounds **6c, 6e, 7, 8a, 8d** screened for *in-vitro* anti-inflammatory activity **(Table 4)** by using Diclofenac Sodium as standard. Compounds **6c** and **8a** have shown good anti-inflammatory activity.

Table-1: Characterization data of the synthesized compounds



6(a-e)



8(a-e)

S. No.	C.C.*		Molecular Formula	M.wt.	% Yield	m.p. (°C)	R_f Value	Solvent system
7.	6a	-O-C_6H_5	$C_{12}H_8N_2OS$	228	68 %	106	0.58	PE:EA* 3:1
8.	6b	-O-C_6H_4-2-CH_3	$C_{13}H_{10}N_2OS$	242	65 %	100	0.60	PE:EA 3:1
9.	6c	-O-C_6H_4-3-CH_3	$C_{13}H_{10}N_2OS$	242	78 %	72	0.57	PE:EA 3:1
10.	6d	-O-C_6H_4-4-CH_3	$C_{13}H_{10}N_2OS$	242	90 %	112	0.63	PE:EA 3:1
1.	6e	-NHC_6H_4-4-$COCH_3$	$C_{14}H_{11}N_3OS$	269	67 %	270	0.47	PE:EA 2:1
2.	8a	-Morpholine	$C_{16}H_{15}FN_4OS$	330	80 %	218	0.38	PE:EA 2:1
3.	8b	-Piperazine	$C_{16}H_{16}FN_5S$	329	88 %	210	0.35	PE:EA 2:1
4.	8c	-NH-2-Pyridine	$C_{17}H_{12}FN_5S$	337	85 %	300	0.24	PE:EA 3:2
5.	8d	-NHC_6H_4-4-NO_2	$C_{18}H_{12}FN_5O_2S$	381	58 %	150	0.40	PE:EA 2:1
6.	8e	-NHC_6H_4-2-NO_2	$C_{18}H_{12}FN_5O_2S$	381	63 %	214	0.74	PE:EA 2:1

*C.C. * = Compound Code, PE: EA* =Petroleum Ether: Ethyl Acetate*

Table-2: Spectral data of synthesized compounds.

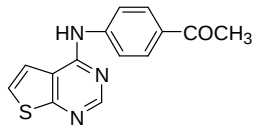
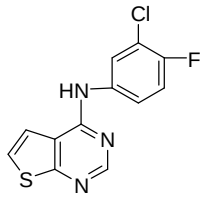
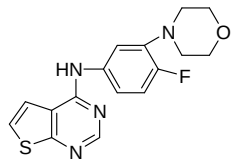
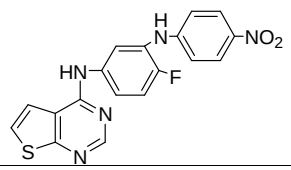
Code	Structure	IR Data (cm^{-1})	NMR data (ppm)
6e		3305.21 cm^{-1} (C-H Str Aromatic) 1510.31 cm^{-1} (C=C Str Aromatic) 2920.32 cm^{-1} (C-H Str Aliphatic) 1178.55 cm^{-1} (C-O-C Str) 2400 cm^{-1} (C-S str) 1663.31cm^{-1}(C=N Str) 1274.59 cm^{-1} (C-N str) 3335.56 cm^{-1}(NH Str Secondary Amine)	
7		1500 cm^{-1} (C=C Str Aromatic) 3230.87 cm^{-1} (C-H Str Aromatic) 1057.03 cm^{-1} (C-F Str) 717.54 cm^{-1} (C-Cl Str) 3444.50 cm^{-1} (NH Str Secondary Amine) 1677.15 cm^{-1}(C=N Str) 1398.50 cm^{-1} (C-N Str)	
8a		1517.31 cm^{-1} (C=C Str Aromatic) 3113.21 cm^{-1} (C-H Str Aromatic) 1677.19 cm^{-1} (C=N Str) 1399.05 cm^{-1} (C-N Str) 3354.52 cm^{-1} (NH Str Secondary Amine) 1251.09 cm^{-1} (C-F Str) 1159.76 cm^{-1} (C-O-C Str) 2325 cm^{-1} (C-S Str)	1H singlet at 9.804 δ –NH (Aromatic) 6H multiplet at 7.402 δ -8.560 δ (aromatic protons) 8H multiplet at 3.284 δ -3.508 δ (Aliphatic protons)
8d		3481.53 cm^{-1} (NH Str Secondary Amine) 1469.31 cm^{-1} (C=C Str Aromatic) 1631.83 cm^{-1} (C=N Str) 1300.07 cm^{-1} (C-N Str) 3113.71 cm^{-1} (C-H Str Aromatic) 2357.05 cm^{-1} (C-S str) 1112.56 cm^{-1} (C-F Str) 1444.73 cm^{-1} (NO_2 Str)	

Table-3: Antibacterial evaluation data of the synthesized compounds

Sl. No.	Comp. Code	Zone of Inhibition (Diameter in mm)			
		***Staphylococcus aureus* (+ve)**		***Bacillus subtillis* (-ve)**	
		50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
01.	6c	4.4 mm	5.0 mm	3.6mm	4.3mm
02.	7	3.4 mm	4.6 mm	3.3 mm	3.6 mm
03.	6e	4.3 mm	5.2 mm	4.0 mm	4.5 mm
04.	8a	3.5 mm	4.5 mm	3.6mm	4.2 mm
05.	8d	3.2mm	4.0 mm	4.5 mm	5.3 mm
06.	Control	3 mm	3.5 mm	1.5 mm	2.2 mm

Standard: Ampicillin = 8.5 mm at 50 µg/ml and 9.5 mm at 100 µg/ml

Table-4: Protein denaturation activity

Comp. Code	Absorbance at 660 nm	% Inhibition of denaturation
6c	0.078	95%
6e	0.064	60%
7	0.067	67.5%
8a	0.080	100%
8d	0.064	60%
Control	0.040	-
Diclofenac Sodium	0.079	***97.5%***

CONCLUSION

A series of 4-substituted-thieno[2,3-*d*]pyrimidine derivatives have been synthesized and characterized successfully. The newly synthesized compounds have been screened for their antiiflammatory activity using the model of inhibition of denaturation of protein and antibacterial activities. In this perspective a wide range of substituents with appropriate substituent groups should be explored in order to optimize the basic thienopyrimidine lead molecule for the development of medicinal property of this class of molecules.

Acknowledgements
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