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Synthesis of 2,3-disubstituted thieno(2,3-*d*)pyrimidines for antibacterial and pharmacological activity

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ABSTRACT

We have synthesized various substituted thieno(2,3-d)pyrimidines, first by synthesizing 2-aminothiophene-3-carboxamide(1), subsequently treating with chloroacetylchloride, 2-(chloroacetamido)thiophene-3-carboxamide(2) was obtained followed by cyclisation, 2-(chloromethyl)thieno(2,3-d) pyrimidine-4one(3) was obtained. The nucleophylic substitution of compound 2-(chloromethyl)thieno(2,3-d)pyrimidine-4one with various aromatic amines, aminophenol gives substituted thienopyrimidines(4a-j).The structure of new compounds were established on the basis of spectral and elemental analysis. The title compounds were screened for antibacterial (agar diffusion method) and antioxidant activity (DPPH scavenging method) and inhibition of denaturation of protein. Most of the compounds have shown promising activity.

Keywords: thieno(2,3-*d*)pyrimidines, bioisosterism, anti-bacterial activity, anti-oxidant activity.

INTRODUCTION

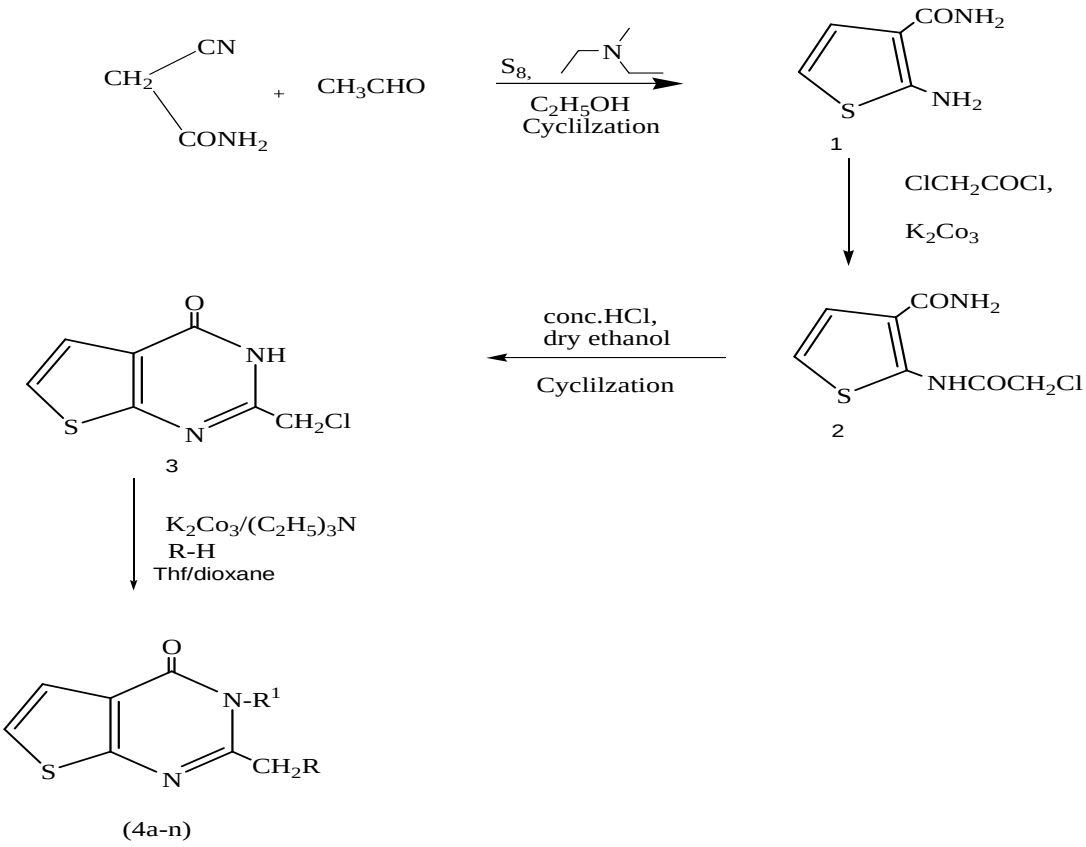
Pyrimidine derivatives play a vital role in many biochemical processes and the transformation. This ring system is present in cytosine, adenine, guanine and thiamine, which form a part of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), vitamins and co-enzymes and other purines.

Pyrimidines have been widely employed in the design of biologically active agents and compounds containing fused pyrimidines have attracted attention in the past few years owing to their wide range of pharmacological activity. Amongst its fused bicyclic, the thienopyrimidines are of great importance because of their remarkable biological activities. To achieve the above mentioned target, scientists have utilized the concept of bioisosterism. It has been defined as group of molecules, which have chemical and physical similarities producing broadly similar biological properties. It is now known that many heterocycles, when appropriately substituted exhibits bioisosterism. Thienopyrimidines occupy a special position among these as these are the structural analogs of biogenic purines. It has been well established, that thienopyrimidines are bioisosteres of quinazolines.

Condensed thienopyrimidines exhibit interesting biological activity like anticancer[1-4], antiviral[5,6], antimicrobial[7-9], analgesic and anti-inflammatory [10,11], anticonvulsant [12], DHFR inhibitors[13], thymidine phosphorylase inhibitors [14], phosphodiestrase IV inhibitors [15], VEGFR-2 kinase inhibitors[16], tyrosine kinase inhibitors, GnRH receptor antagonistand adenosine receptor binding properties.

In the current literature survey, it has been observed that drug designed by molecular modification is more rational and productive in discovering a new drug, consequently the need to synthesize a new molecule as potential medicinal agent is relevant today. Here in, we are presenting synthesis of novel 2,3-disubstituted thieno(2,3-*d*)pyrimidines[4a-n] and their antibacterial, antioxidant and anti-inflammatory activities.

MATERIALS AND METHODS



The melting point of the compounds was taken in open capillaries and is uncorrected. The infrared spectrum was recorded using KBr as the medium, utilizing SHIMADZU Infrared spectrophotometer.^{1}H NMR Were recorded from Astra- Zeneca Pharma India Ltd. Bangalore. All the reactions were monitored using thin layer chromatography (TLC) using a glass plate coated with Silica Gel G or GF $_{254}$and spots were visualized either by iodine vapour or by irradiation with ultraviolet light (254 nm).

Synthesis of 2-aminothiophene-3-carboxamide (1):

Acetaldehyde (0.04mol, 1.76gm / 2.25ml), cyanoacetamide (0.04 mol, 3.36gm), sulphur (0.04 mol, 1.28gm) and ethanol (40ml) were taken in conical flask and warmed up to 40^{o}-50^{o}C. Then triethylamine (4ml) was added drop wise with constant stirring until the sulphur went into the

solution. Stirring was continued for 1 hr, till the solid separated. Then it was cooled to room temperature and filtered. The product was dried and recrystallized from ethanol.

Synthesis of 2-(2-chloroacetamido) thiophene-3-carboxamide(2):
2-aminothiophene-3-carboxamide from part one (0.142g, 1mM) and potassium carbonate (0.276g, 2mM) in dry DMF was stirred at room temperature for half an hr. Chloroacetylchloride (0.112g/0.15ml, 1mM) was added in reaction mixture at 0^0c and, stirred for 3 hr. The mixture was poured in water with stirring. The product obtained was filtered, washed with water, dried and recrystallized from DMF: Water. The % Yield of pure product was 50.25 %.The R_f value was found to be 0.48[mobile phase Ethyl acetate: n-Hexane (2:1)]. The melting point was determined and found to be158^0c.

Synthesis of -2-(chloromethyl)thieno(2,3-d)pyrimidin-4(3H)one(3):
2-(2-chloroacetamido)thiophene-3-carboxamide (0.218g,1mM) obtained from part two and concentrated hydrochloric acid (0.2ml) in dry ethanol was heated under reflux for 5 hr and left to cool at room temperature overnight. The solid formed was filtered, dried and recrystallized from ethanol: water. The % Yield of pure product was 78%. The R_f value was found to be 0.45[mobile phase, Ethyl acetate: n-Hexane (2:1)]. The melting point was determined and found to be 275^0c.

Synthesis of 2-(substitutedphenylamino)methyl)thieno (2, 3-d) pyrimidin-4(3H)-one (4a-h):
To the solution of product 2-(chloromethyl)thieno[2,3-d]pyrimidin-4(3H)-one (0.2g,1mM) obtained from part 3 in dioxane, triethylamine (0.1g/0.136ml,1mM) and substituted aromatic amines(1mM) were added. Refluxed on oil bath for 8 hr. Reaction mixture was cooled to room temperature and poured into ice cold water. The solid obtained was filtered, washed with water and recrystallized using DMF.

Synthesis of 2-(substituted) methyl) thieno (2, 3-d) pyrimidin-4(3H)-one (4i-k):
To the solution of product 2-(chloromethyl)thieno[2,3-d]pyrimidin-4(3H)-one (0.2g,1mM) obtained from part 3 in dioxane, morpholine (0.609g/0.675ml, 7mM)/ piperidine (0.595g/0.7ml,7mM)/ Nmethylpiperazine (0.7g/.77ml,7M) was added. The reaction mixture was refluxed for 18 hr. Reaction mixture was cooled to room temperature and poured into ice cold water. The solid obtained was filtered and washed with water. Recrystallized using DMF: water mixture.

Synthesis of (hydroxyphenylamino)methyl)thieno(2,3d)pyrimidin-4(3H)-one(4i-n):
Aminophenol (0.132gm, 1.2mM) in THF (25ml) was completely solubilised under inert (N_2) atmosphere. To this potassium carbonate (0.55gm, 4mM) was added and stirred for 30 min. Later the solution of 2-(chloromethyl) thieno [2, 3-*d*] pyrimidin-4(3H)-one (0.2g, 1mmol) in THF was added drop by drop .The reaction mixture was refluxed for 6-8 hr. The progress of reaction was monitored by TLC. The solvent was removed from reaction mixture under reduced pressure in rotary evaporator. The compound was extracted in ethyl acetate. The ethyl acetate layer was washed with water and dried over anhydrous sodium sulphate. The ethyl acetate was evaporated under reduced pressure to get light brown solid.

IR spectra in KBr (cm^{-1}): Compound 4c :- N-H straching -3456.55, C-H straching aromatic-3192.30, C-H straching aliphatic-2850, C=O straching -1 674.21, C=N starching- 1591.33, NO_2 straching- 1550.82 C-N starching- 1336.31

^{1}HNMR data: Compound 4c :- 1H doublet at 8.13-8.16δ-CH-aromatic, 1H doublet at 7.58-7.61 δ -CH-aromatic, 2H singlet at 3.30 δ-aliphatic CH_2,1H singlet at 9.1 δ-NH- amide, 2H doublet at 6.55-6.58 δ -CH-aromatic benzene, 1H singlet at 8.95 δ-NH- amine,1H triplet at 6.98-7.06δ -CH-aromatic.

Anti-bacterial activity

Agar diffusion method

In our current study, the antimicrobial activity was carried out by the agar diffusion method. The micro organisms used were *Staphylococcus aureus* (G +ve) and *E.Coli* (G -ve). Here responses of organisms to the synthesized compounds were measured and compared with the response of the standard reference drug. Each test compound was dissolved in DMF to get a concentration of 50µg/ml. The standard reference drug used in the present work was Ampicillin.

Inhibition of denaturation of protein[17,18]

Bovine serum albumin (Merck Limited), Ibuprofen and all other chemicals are of analytical grade. The test compounds were dissolved in minimum amount of DMF and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solution was less than 2.5%. Test solution (1 ml) containing different concentrations of drug was mixed with 1 ml of 1 mM albumin solution in Phosphate buffer and incubated at (27) ºc for 15 min. Denaturation was induced by keeping the reaction mixture at (60) ºc in a water bath for 10 min. After cooling the turbidity was measured at 660 nm. Percentage inhibition of denaturation was taken. The percentage of inhibition is calculated from the following formula,

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

Where, Vt – Absorbance of test solution,
Vc – Absorbance of control

Antioxidant activity-DPPH radical scavenging activity:[19,20]

In Vitro Free Radical Scavenging activity(Reduction of DPPH ions)

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging effect was carried out according to the method first employed by Blois. Compounds of different concentrations were prepared in distilled methanol, 1 mL of each compound solutions having different concentrations (10, 25, 50, 100, 200 and 300 µM) were taken indifferent test tubes, 4 mL of 0.1 mM methanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound, and methanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV–visible spectrophotometer (Shimadzu 160A).

The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula:

$$\text{Radical scavenging activity} = [\ (A_0 - A_1/A_0) \times 100]$$

A_0 is absorbance of the control
A_1 is absorbance of the compound.

The radical scavenging activity of butylated hydroxyl toluene was also measured and compared with that of the different synthesized compound. The compound concentration providing 50% inhibition (IC_{50}) was calculated from the graph of RSA percentage against compound concentrations.

RESULTS AND DISCUSSION

Table-1. Properties of synthesized compounds (a-e)

S.N.	Compounds code	R=(aromatic amines or alcohols)	% yield	Melting Point(^{0}c)	R_f Value
1	4a	Phenylamino	67	235	0.55
2	4b	2-nitrophenyl amino	80	265	0.57
3	4c	3-nitrophenyl amino	88	245	0.55
4	4d	4-nitrophenyl amino	85	238	0.57
5	4e	2-chlorophenyl amino	68	238	0.6
6	4f	3-chlorophenyl amino	65	234	0.55
7	4g	4-chlorophenyl amino	50	220	0.55
8	4h	3-chloro-4-flurophenyl amino	54	198	0.57
9	4i	morpholino	60	268	0.46
10	4j	piperazinyl	48	168	0.49
11	4k	Piperidinyl	43	189	0.48
12	4l	2-hydroxyphenylamino	58	176	0.67
13	4m	3-hydroxyphenylamino	64	276	0.66
14	4n	4-hydroxyphenylamino	66	273	0.67

Biological activity

The antibacterial screenings revealed that some of the tested compounds showed good inhibition at50µg/0.1ml concentration. The antibacterial screening indicated that among the tested compounds 4b,4h and 4e showed excellent activity against the tested bacterial strains namely *S. aureus* and *E.coli* (Table 2). The compounds 4c, 4d, 4f, 4g were found to be moderately active. The compounds 4i, 4j, 4l-n showed least activity in the series.

Table-2. Anti-bacterial activity

Sr. No.	Compound Code	Zone of Inhibition (Diameter in mm)			
		Staphylococcus aureus Gram (+ve)		*E.coli* Gram (-ve)	
		50µg	**100** µg	50µg	100µg
01	4a	05	07	05	08
02	4b	08	10	10	11
03	4c	05	08	07	09
04	4d	06	09	06	08
05	4e	11	12	08	10
06	4f	06	10	06	10
07	4g	07	11	07	10
08	4h	10	12	10	13
09	4i	04	08	04	08
10	4j	05	08	05	08
11	4k	05	10	04	09
12	4l	04	07	04	07
13	4m	04	06	03	07
14	4n	04	06	03	06
15	Ampicillin	10	14	11	16

Pharmacological Activity- In-vitro inhibition of denaturation of protein screening revealed that some of the tested compounds Showed good activity. The results indicated that among the tested compounds4b and 4e showed excellent activity (Table-3). The compounds4a and 4k showed least activity in the series. The remaining compounds were found to be moderately active.

Table -3. Protein denaturation Activity

Conc in µM	STD (Ibuprofen)	4a	4b	4e	4k
25	15.24	7.92	14.89	15.01	3.9
50	25.05	11.34	20.80	22.01	7.8
100	45.03	14.89	26.95	30.37	13.83
200	75.17	20.80	39.95	48.34	29.78
300	98.81	26.95	51.53	60.04	39.11
IC_{50}	116	360	289	214	400

Compounds were also screened for anti-oxidant activity (DPPH scavenging activity). Five of them showed somewhat inhibition. Among them compound 4l and 4m showed good inhibition at lower concentration (Table-4).

Table-4.Anti-oxidant activity

Conc. in µM	STD (BHT)	4a	4b	4e	4l	4m
10	2.27	13.85	20.43	20.31	13.85	26.16
25	4.89	15.05	22.23	23.53	16.36	26.52
50	15.41	17.54	23.53	25.32	22.34	29.39
100	38	18.04	25.32	32.49	30.1	39.55
200	76.03	21.62	28.91	43.1	43.1	51.97
300	99.88	25.08	30.7	51.97	63.39	60.21
IC_{50}	132	400	350	280	234	184

CONCLUSION

Series of 2,3Disubstituted analogues of thieno(2,3-*d*)pyrimidine have been synthesised and screened for their inhibition of denaturation of protein, antibacterial and antioxidant activities. In this connection, 2-((phenylamino)methyl) thieno[2,3-d]pyrimidin-4(3H)-one bearing electron withdrawing group demonstrated good antibacterial and anti-inflammatory activity. Whereas molecules attached with electron donating groups exhibited better antioxidant activity. In this perspective a wide range of substituents with appropriate electron donating or withdrawing groups should be explored in order to optimize the basic thienopyrimidine lead molecule for the development of medicinal property of this class of molecules.

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REFERENCES

[1] J Adrian, A Khatereh , K Wendy , A Sonia, J Stewart, B Gary,| S Irina, *J Med Chem* , **2008**, 51, 5522–32.

[2] LDJennings , SL Kincaid , DW Yanong ,G Krishnamurthy ,CF Beyer , *Bioorg med chem lett* ,**2005**, 15, 4731-35.

[3] YDWang ,S Johnson,D Powell, McGinnis, M Miranda,SK Rabindran, *Bioorg med chem lett* ,**2005**, 15, 3763-66.

[4] T Horiuchi,J Chiba, K Uoto,T Soga, *Bioorg med chem lett*, **2009**, 19, 305–08.
[5] A Angell,C McGuigan,LG Sevillano,R Snoeck,G Andrei,ED Clercq,J Balzarini, Bicyclic anti-VZV nucleosides: *Bioorg med chem lett* , **2004**, 14,2397–99.
[6] C Mcguigan,A Brancale,B Algain,S Pascal, R Benhida,JL Fourrey, *Bioorg med chem lett*,**2001**, 11,2507–10.
[7] M Hossain,MD Khandekar,R Mizanur,K Hossain, ARahim,MI Hossain,*Acta pharma*, **2006**,56,441–50.
[8] RV Chambhare, BG Khadse,AS Bobde,RH Bahekar, *Eur j med chem*, 2003, 38, 89- 100.
[9] S Yousieff,BE Bayoumy, *J pharm sci*, **1990**, 31(1-4), 67.
[10] V Alagarsamy,S Vijayakumar, VR Solomon, *Biomed Pharmacotherapy*, **2007**, 61,285-91.
[11] V Alagarsamy,S Meena,KV Ramseshu,VR Solomon,K Thirumurugan, K Dhanabal , M Murugan , *Eur j med chem.*, **2006**,41, 1293–1300.
[12] M Santagati , Modica M, Santagati A, Russo F, Spampinato S. *Pharmazie* **1996**; 51(1): 7-11.
[13] IO Donkor , LI Hui ,SF Queener, *Eur j med chem*, **2003**, 38, 605-11.
[14] LP Melissa,WC Guida,TE Jackson,AN Jason,LG Patricia,JC Juarez JC, *Bioorg med chem lett* **2003**, 13, 107–110.
[15] AK Chakraborti,B Gopalakrishnan,ME Sobhia,A Malde, *Bioorg med chem lett* **2003**,13,1403–1408.
[16] JM Michael,SB Jean,MC Jeffery,AC Beth AC,LD Jonathan, *Bioorg med chem lett* **2004**,14,21-24.
[17] MM Vijaykumar,TS Nagaraja,H Shameer,E Jaychandran,GM Sreenivasa, *J pharm sci res* **2009**, 2,83-92.
[18] LVG Nargund,J Robin,YSR Reddy YSR, ECV.Arzneim –*Forsch. /Drug Res*, **1994**, 44,156-58.
[19] VH Kumar,N Naik, *Eur j med chem.* **2010**, 45,2–10.
[20] MM Hossain,SK Shaha ,F Aziz, *Bangladesh med res counc. bull* **2009**, 35,49-52.