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Spectral and Biological analysis of Thieno [2, 3-d] pyrimidin-4-one

Rovin[a], Dharmendra Kumar[b], Vikas Kumar[c,] Leeladhar Singh[d]
[a] Department of Physics, Monad University, Ghaziabad, India
[b] Department of Applied Physics, IIMT Engineering College, Meerut, India
[c] Department of Applied Chemistry, IIMT Engineering College, Meerut, India
[d] Department of Physics, Monad University, Ghaziabad, India

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Abstract

Thieno [2,3-d] pyrimidin-4-one is synthesized by 2-amino-3-carboethoxythiophene and fomamide and its structure was also established using FTIR, UV-Vis and 1H-NMR spectroscopic method. The synthesized compound was also tested for antimicrobial activity against Escherichia coli, Bacillus subtilis and Staphylococcus aureus and fungus Candida albican, Aspergillus niger and Candida krusei.

1. Introduction

Fused pyrimidines have also been attracted a considerable interest in medicinal chemistry research due to their versatility and a broad bioactive potential. Thienopyrimidine is among those fused pyrimidines found to have a wide variety of pharmacological and biological applications. Since last four decades research has been focused on the design and synthesis of novel thienopyrimidines as medicinal agents, a large number of reports have been documented on thienopyrimidines as they found to exhibit a variety of biological activities such as antimicrobial [1,2], anti-inflammatory [3,4], anticancer [5,6], analgesic [7,8]. In continuation of our research program to find out bioactive thienopyrimidines, the present work is an effort towards the synthesis of thieno [2,3-d]pyrimidin-4-one and characterized by FTIR[9,10], 1H-NMR [11,12], Raman spectroscopy [13,14], UV-Visible spectroscopy [14,15] etc.

2. Methods and Materials

2-amino-3-carboethoxythiophene (2mmol) and fomamide (20 mL) was heated under reflux for 3 hours and then left to cool to room temperature overnight. The solid was filtered washed with water. Dried and recrystallized from ethanol. The melting point of thieno [2,3-d]pyrimidin-4-one is 245 °C, the yield is 95 %.

All the reagents and solvents were generally received form commercial supplier. Reactions were done in dried glassware. Melting points were taken in open capillaries by thermonic melting point apparatus, (Campbell Electronic Mumbai, India) and are uncorrected. The purity of the newly synthesized compounds was checked by thin layer chromatography (TLC) on silica gel-G coated plates by using different solvent systems. Infrared (IR) spectra were determined on Bruker IFS-66 FTIR (Bruker Bioscience, USA) using KBr pallets and wave number (ν) was reported in cm^{-1}. The ^{1}H-NMR spectra were taken on Jeol GSX -300 FT NMR (Jeol, Tokyo, Japan) in $CDCl_3$ or DMSO-d_6, and chemical shifts (δ) are given in ppm. Tetramethylsilane (TMS) was used as internal reference standard. Mass spectra were recorded on Spec Finnigan Mat 8230 MS. The carbon, hydrogen and nitrogen analysis were performed on Carlo Erba-1108 (Carlo Erba, Milan, Italy), and the results were found within ± 0.4% of the theoretical values. The electronic spectra (UV-Vis) were recorded on a Perkin-Elmer Lambda 15 UV-Vis spectrophotometer, using 10^{-3} mol·dm^{-3} solutions in DMF.

Corresponding Author,
E-mail address: drdksisodia@gmail.com
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3. Antimicrobial activity

The antimicrobial activity was assayed in vitro by the two fold broth dilution[16]against bacteria Escherichia coli, Bacillus subtilis and Staphylococcus aureus and fungus Candida albican, Aspergillus niger and Candida krusei. The minimal inhibitory concentrations (MIC, µg/ml) were defined as the lowest concentrations of compound that completely inhibited the growth of each strain. All compounds, dissolved in dimethylsulfoxide, were added to culture media .Mueller Hinton Broth for bacteria and Sabouraud Liquid Medium for fungi to obtain final concentrations ranging from 125 µg/ml to 1.592 µg/ml. The amount of dimethylsulfoxide never exceeded 1% v/v. Inocula consisted of 5.0 $x10^4$ bacteria/ml and 1.0 $x10^3$ fungi/ml. The MICs were read after incubation at 37 °C for 24 h (bacteria) and at 30°C for 48 h (fungi). Media and media with 1% v/v dimethylsulfoxide were employed as growth controls. Chloroamphenicol and fluconazole were used as reference antibacterial and antifungal drugs, respectively.

To detect the type of antimicrobial activity, subcultures were performed by transferring 100 µl of each mixture remaining clear in 1 ml of fresh medium. The minimal bactericidal concentrations (MBC, µg/ml) and the minimal fungicidal concentrations (MFC, µg/ml) were read after incubation at 37 °C for 24 h and at 30 °C for 48 h, respectively.

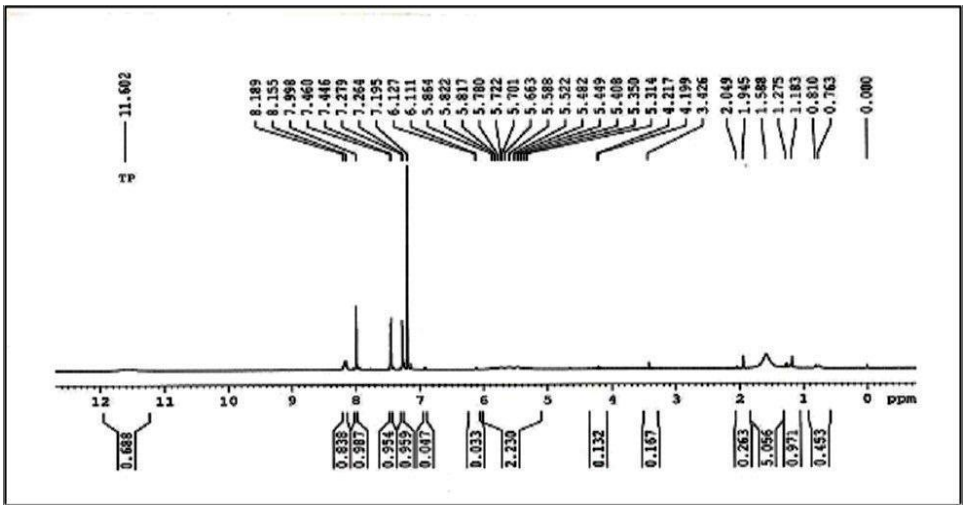


Fig: 1. H-NMR Spectroscopy of thieno [2, 3-d]pyrimidin-4-one

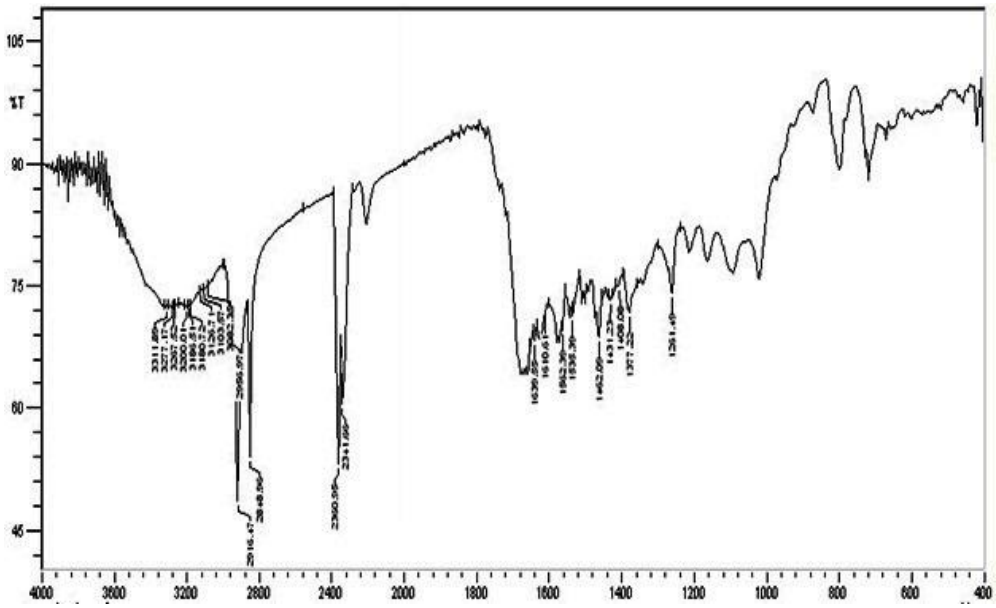


Fig: 2. FTIR-Raman Spectroscopy of thieno [2, 3-d] pyrimidin-4-one

Table: 1. Vibrational assignments of fundamental frequencies (cm^{-1}) of Thieno[2,3-d]pyrimidin-4-one.

Species	Observed Frequencies (cm^{-1})		Calculate Frequencies (cm^{-1})	Assignment
	FTIR	Raman		
a^I	3412(ms)	-	3458	N-H stretching
a^I	3250(s)	-	3260	C-H stretching
a^I	3173(s)	-	-	C-H stretching
a^I	-	3068(s)	-	C-H stretching
a^I	2995(w)		-	C-H stretching
a^I	2901(w)	-	-	C-H stretching
a^I	1664(s)	-	1690	C=O stretching
a^I	1602(w)	-	-	C=O stretching
a^I	-	1551(s)	-	C=O stretching
a^I	1521(ms)	-	1565	C=N stretching
a^I	1489(ms)	-	-	C=N stretching
a^I	1404(w)	-	1465	C=C stretching
a^I	-	1375(w)	-	C=C stretching
a^I	-	1345(w)	-	C=C stretching

a^I	1311(s)	-	1365	C-N stretching
a^I	1214(w)	-	1275	N-H in plane bending
a^I	1138(s)	-	1138	C-H in plane bending
a^I	1116(ms)	-	-	C-H in plane bending
a^I	-	1074(w)	-	C-H in plane bending
a^I	-	1051(w)	-	C-H in plane bending
a^I	1024(w)	-	-	C-H in plane bending
a^I	912(w)	-	968	C-C in plane bending
a^I	680(w)	-	664	C-S-C in plane bending
a^I	-	615(s)	-	C-S-C in plane bending
a^I	570(w)	-	564	C-C-H in plane bending

Table: 2. 1H-NMR data of Thieno [2, 3-d] pyrimidin-4-one.

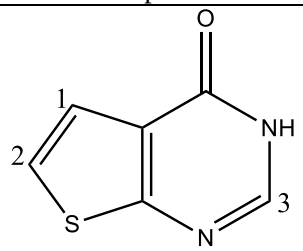
Compound	δ/ ppm	Assignments
	7.26-7.27	d, (*J=7.1Hz*)H of CH(H1)
	7.44-7.66	d, (*J=8.1Hz*)H of CH(H2)
	7.99	s, (*J=4.1Hz*)H of CH(H3)
	8.12	s, H of NH

Table: 3. Electronic spectral data in 95% ethanol and DMF, λmax(nm) / εmax (10^3 $mol^1.dm^3.cm$)

Solvent	Thieno[2,3-d]pyrimidin-4-one			
	I	II	III	IV
Ethanol	201.5/2.61	252.5/1.63	396.5/2.21	256.00/2.82
DMF	-	261.6/2.8164	-	402.0/1.4227

Table: 4. Minimal inhibitory concentration (MIC) µg/ml of Thieno [2, 3-d] pyrimidin-4-one. against tested bacterial and fungal strains

Compound No.	Minimal inhibitory concentration (MIC) µg/ml					
	E. coli	B. subtilis	S .aureus	C. albicans	A. niger	C. krusei
a^I	6.25	3.125	12.5	3.125	3.125	1.592
Chloroamphenicol	12.5	6.25	12.5	-	-	-
Fluconazole	-	-	-	6.25	12.5	3.125

4. Result and Discussion

Spectral analysis

The heteroaromatic structure shows the presence of C-H stretching, in-plane bending vibrations in the regions 3200-3000 cm^{-1} and 900- 1200 cm^{-1} respectively. In this region the bands are not affected appreciably by the nature of the substituents. The FTIR bands at 3173, 3125, 2995, 2910, and 2870 cm^{-1} and FT-Raman bands at 3160, 3110, 3080, 2926 and 2863 cm^{-1} in Thieno[2,3-d]pyrimidin-4-one is assigned to C-H stretching modes. The bands at 1138, 1076, 1076, 1016 cm^{-1} have been assigned to C-H in-plane bending vibrational modes.

The IR and Raman bands identified at 3412 and 3382 cm^{-1} are assigned to N-H stretching mode. The N-H in-plane bending vibration is found at 1220 cm-1.

The C=N stretching frequencies in the Raman spectrum of crystalline Thieno[2,3-d]pyrimidin-4-one occur in the range 1552-1490 cm^{-1}. In the present investigation, the Raman bands observed at 1552, 1489 cm^{-1} have been assigned to C=N stretching vibrations. The very strong IR peak and the strong Raman peak observed at 1551 cm^{-1} is assigned to C-N stretching mode.

If a compound contains the carbonyl group the absorption caused by C-O stretching is generally strongest. In Thieno[2,3-d]pyrimidin-4-one identified the C-O stretching frequency at 1662 and 1602 cm-1.

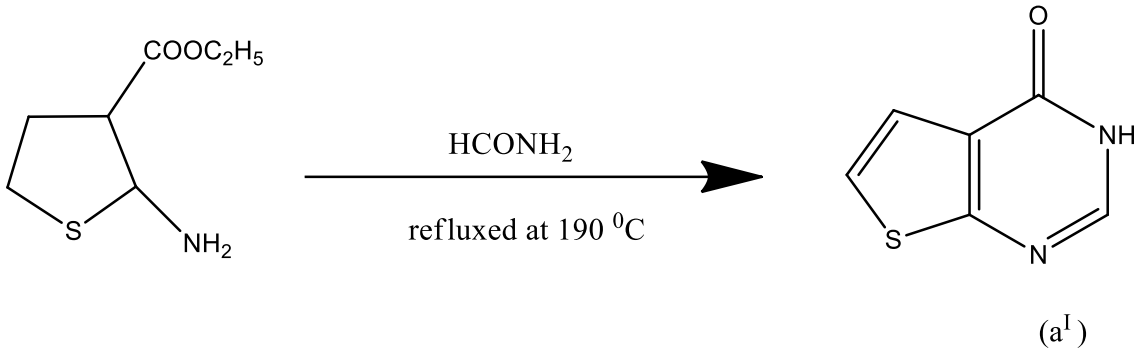
The carbon-carbon stretching vibrations of the title compound have been observed at 1600, 1590, 1580 and 1524 cm^{-1}. The medium Raman bands identified at 912 and 862 cm^{-1} have been assigned to C-C in-plane bending.

The carbon-sulphur stretching vibrations of the title compound have been observed at 680 cm-1. The medium Raman bands identified at 615 cm-1 have been assigned to C-S in-plane bending.

In the ^{1}H-NMR spectra, the singlet signal at δ8.10 ppm is assigned to NH based on the position of this peak in the spectrum of the parent pyrimidine-4-one molecule. The assignment of the peak at δ7.9 ppm of H (3) proton of CH of pyrimidine molecule is obtained. Two doublet signal of H (1) and H (2) of 2-thiophene are found.

UV-Vis absorption spectra of thieno [2,3-d]pyrimidin-4-one after the continuous prolonged irradiation (0, 5, 15, 30, 45 and 60 min) with UV-A light. Both the absorption maxima (λ_{max}= 348 nm and λ_{max}= 355 nm) decrease, and a slight bathochromic shift have been detected, at the end of any particular UV-irradiating period. The log values of the

absorbance maxima plotted against irradiation time yielded a linear plot, suggesting the involved kinetics to be probably of pseudo-first order, depending on the thieno [2,3-d]pyrimidin-4-one concentration only



(a^I)

Fig: 3. Formation of thieno [2, 3-d] pyrimidin-4-one

5. Antimicrobial activity (Minimal inhibitory concentration)

Antibacterial activity of thieno[2,3-d]pyrimidin-4-one (a^I) and standard drug, chloroamphenicol, was carried out at a concentration 250 µg/ml against *E. coli ATCC 25922*, *B. subtilis ATCC 1633* and *S. aureus ATCC 25923*. Results show the varying degree of antibacterial activity of all the compounds tested (Tables 1). From the results obtained, it is clear that thieno[2,3-d]pyrimidin-4-one exhibited less activity against *E. coli ATCC 25922*, *B. subtilis ATCC 1633* than chloroamphenicol but *S. aureus ATCC 25923* displayed antibacterial property comparable to the reference drug.

The compound thieno[2,3-d]pyrimidin-4-one (a^I) along with reference drug, fluconazole, were also tested for antifungal activity at a concentration of 250 µg/ml against *C. albicans ATCC 2091*, *A. niger ATCC 9029* and *C. krusei ATCC 6518*, and it is found that synthesized is showed very weak or moderate active as compared to standard drug.

6. Conclusion

Thieno[2,3-d]pyrimidin-4-one established using FTIR, UV-Vis and ^{1}H-NMR spectroscopic method. Vibrational and electronic spectra confirmed the synthesized compound, thieno[2,3-d]pyrimidin-4-one . The compound was tested for its *in vitro* antimicrobial activity and its activity against bacteria *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* and fungus *Candida albican*, *Aspergillus niger* and *Candida krusei*compared to chloramphenicol and fluconazole, respectively.

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