Synthesis and antimicrobial activities of some novel thieno [*2,3-d*]- Pyrimidin-4(3*H*)-One derivatives

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Abstract
Pain and inflammation are simultaneous responses in bacterial infections. In current clinical practice, the agents like antimicrobial drug are prescribed concurrently. A $POCl_3$ catalyzed, efficient, one-step and solvent-free synthesis of novel thieno [2, 3-d] pyrimidin-4(3H)-one derivatives from 2-amino-4,5-substitutedthiophene-3-carbonitrile has been developed using various aliphatic acid under conventional heating and microwave irradiation. The formation of compounds was confirmed using elemental analysis and spectroscopic techniques like FTIR, ^{1}H NMR and Mass spectroscopy. All synthesized compounds have been screened for their antimicrobial activity against *Escherichia coli* (Gram –ve strain), *Bacillus subtilis* (Gram +ve strain) for antibacterial activity and antifungal activities against *Aspergillus niger* and *Candida albicans.* The result showed that synthesized compounds exhibit weak, moderate and good antimicrobial activity. It was observed that the compounds 2a, 2c, 2d, 2e, 2f, 2g, 2j and 2k showed good antimicrobial activity whereas compounds 2b, 2i, 2j showed significant antimicrobial activity compared with standard drug Streptomycin and Amphotericin B respectively.

Keyword: $POCl_3$, Thieno[2, 3-d]pyrimidin-4 (3H)-one, Antimicrobial activity, Streptomycin and Amphotericin B.

Introduction

Medication revelation is ceaseless and iterative process, which begin with the recognizable proof of lead atom of wanted natural activity(lead age and closures with the streamlining of this lead(lead advancement) for choice of new hopeful particle in sedate improvement.[1] The attention to synthetic, physical physiological, biochemical properties, receptor locales, SAR and stereochemistry and so on is extremely huge in sedate plan for the fruitful advancement of medication particle.[2] Since sedate plan is a coordinated for building up the train which forecasts a time of adjusted medication, a medication lacking symptom. it looks to clarify impacts of natural structure or its physicochemical properties included.[3] It examines the procedures by which the medications delivered their belongings; how they respond with the cellular material of inspire a specific pharmacological impact or reaction. how they changed or detoxified, used or disposal by living being.[4] These idea are the building stones whereupon the structure of medication configuration in assembled. The various new advancements have been created and connected in tranquilize innovative work (R&D) to abbreviate the examination cycle and to decrease the costs.[5] Among them, computational methodologies have upset the pipeline of disclosure and advancement over the most recent 40 year, computational advances for medicate R&D have advanced rapidly, particularly in late decades with the extraordinary improvement of science, biomedicine, and PC ability.[6] The computational instruments have been connected in relatively every phase of medication R&D, which have incredibly changed the system of medication disclosure.[7]

Thiophene containing compounds are well known to exhibit various biological effect. Heterocycles containing the thienopyrimidine moiety are of intrest because of their intresting pharmacological and biological activities.[8-9] They bear structural analogy and isoelectronic relation to purine and several substitutedthieno[2,3-*d*] pyrimidine derivatives shown to exhibit prominent and versatile biological activities [10-11].Over the last two decades, many thienopyrimidines have been found to exhibit a variety of synthesized as potencial anticancer,[12] analgesic,[13] antimicrobial[14-15] and antiviral agents.[16]

Recently, we reported some reviews on pyrimidinethiones[17] and condensed pyrimidines, namely pyrazolo-pyrimidines[18] and furopyrimidines.[19] The work deals with the study of the synthesis, reaction and biological application of thienopyrimidines in veiw of their great importance.in the last decade, thienopyrimidines were review.[20] The three fundamental thienopyrimidines systems are thieno[2,3-*d*]pyrimidine (I), thieno [3,2-*d*] pyrimidine (II) and thieno [3,4-*d*] pyrimidine (III).This article aimed to show the recent novel precursors to synthesize thienopyrimidine derivative and reported their application in pharmaceutical and biological evaluations in the last decade.[21-23] Various synthetic approaches have been utilized for the synthesis of thienopyrimidines. Recently, Bakavoli et al. used molecular iodine as an oxidising agent for the synthesis of thienopyrimidines via an oxidative heterocyclization reaction. However, the synthesis of thienopyrimidine from 2-amino-4, 5-substitutedthiophene-3-carbonitrile requires two steps and solvant-free method to generate a series of thieno [2,3-d] pyrimidin-4(3H)-one derivatives. In recent times, microwave assisted

synthesis of medicinal compounds has gained appreciation among the synthetic chemists due to their improved selectivity, Shorter reaction time, eco-friendliness and superior work-up procedures. Microwave have been used to speed up chemical reactions in the laboratories which led scientists to investigate the mechanism of microwave dielectric heating and to identify the advantages of the technique for chemical synthesis.[24] In the current era, antibiotics and synthetic antimicrobial agents have changed the scenario of the medical field in the treatment of various bacterial and fungal infections. However, occurrence of various drug-resistant microbial strains posed a existing contest to the medicinal chemists.[25] Fused pyrimidines attracted considerable attention because of great practical usefulness, primarily, due to its very wide pharmacological activities. Fused pyrimidine chemistry began in 1776, when Scheele isolated uric acid. Many simple pyrimidines such as pteridines and purines are biologicaly active by themselves and essential components of very important naturally occurring substances (i.e.nucleic acid).Examples of some biologically active pyrimidine derivatives are prazosin, quinethazone, trimethotrexate, folic acid, riboflavin.Since fused pyrimidines are pteridines, pyridopyrimidines, triazolopyrimidines,pyrazolopyrimidines, pyrimidoazepines, furopyrimidines and pyrrolopyrimidines. Thienopyrimidines occupy a special positions among a fused pyrimidines as these are the structural analogs of biogenic purines. The wide range of biological activities of thienopyrimidine derivatives has stimulated considerable research in this field.[25]

Materials and Method

All the chemicals and solvents used were of AR and LR grade, obtained from Loba, Merck and Fisher scientific fine chemicals (Mumbai, India).The progress of reaction was tested on precoated silica gel G plates obtained from Merck ,using the mobile phase toluene and ethyl acetate in 7:3 ratio. Iodine chamber and UV lamp (k =254 nm) were used for visualization of the spots. LABHOSP melting point apparatus was used for measurement of melting points in an capillary tube and are uncorrected. The IR spectra (vmax, cm^{-1}) were recorded on Shimadzu FT-IR IRA-Affinity-1 spectrophotometer as KBr pellet technique. ^{1}HNMR (∂, ppm) spectra were recorded on Bruker Avil-400 MHz spectrophotometer. ^{1}HNMR spectra for synthesized compound were recorded with $CDCl_3$ as solvent. Mass spectra were recorded on water UOLC-TQD (ESI-MS&APCI-MS)

General procedure for preparation of 2-amino-4,5-substitutedthiophene-3-carbonitrile (scheme 1)(1a-1h)

Take a mixture of substituted ketones (1a-1g) (0.01M). malanonitrile (0.01M),sulfur (0.01M) and ethanol (10mL)Were mixed in a conical flask. The reaction mixture was warmed up to 40-50°C on a water bath and then diethylamine (1mL) was added with constant stirring in such a way that the temperature does not exceed 50°C.Stirring was continued for 1-2h till solid crystals gets separated. The reaction mixture was then cooled and kept in a refrigerator. The fine crystals thus obtained were filtered. dried and recrystallized from ethanol to give compounds.

General procedure for preparation of thieno[2,3-d]pyrimidin-4(3H)-one derivatives (scheme 2)(2a-2k)

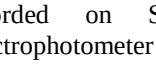
Conventional synthesis

2-Amino-4,5-substitutedthiophene-3-carbonitrile (1a-1h) (1mM) was dissolved in appropriate aliphatic acid (2mL).Then $POCL_3$ (0.2mL) was added drop wise and the reaction mixture has been kept for reflux on a boiling water bath. After completion of the reaction, the mixture was poured on ice-cold water (50mL) and crude precipitates thus formed were filtered washed with 10% sodium bicarbonate solution dried and recrystallized from ethanol.

Microwave assisted synthesis

A mixture of 2-amino-4, 5-substitutedthiophene-3-carbonitrile (1a-1h) (1mM) and alumina (0.5g) were finally crashed and transferred to a glass vial and then phosphorus oxychloride (0.2mL) was added to this mixture. The glass vial was then capped and microwaves were irradiated in a microwave oven SAMSUNG (Self fabricated microwave analyzer) at a power of 180W for 2-4 min. After the completion of reaction, the mixture was poured on ice-cold water (50mL). The precipitated product was filtered and washed with 10% sodium bicarbonate solution to give the desired compounds.

Synthesis of 2, 8 dimethyl-5,6,7,8-ahydrobenzo-[4,5]thieno[2,3-d]pyrimdin-4(3H)-one

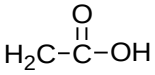
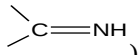
IR(KBr,cm^{-1}): 3413.19 (N-H strech), 1385.66(C-CH_3),  (1600.09),1665.60(C=S), 1134.10(C-S),^{1}HNMR ($CDCl_3$,∂),0.9(-CH_3),4.73 singlet (N-H), 9.3 singlet (Aldehydic R-CH=O), 7.29 triplet (Hetrocyclic amine); MS: m/z 335.21 (M^+, 100%) M^{+2} at 336.

Synthesis of 6-(2-hydroxyphenyl)-2, 5-dimethylthieno [2,3-d] pyrimidine-4(3H)-one

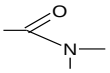
IR(kBr,cm^{-1}): 3413.19 (N-H strech),1214.04 (-OH), 1106.12 (C-S), 1633.7 (C=O), 1666.80(C=N), ^{1}HNMR ($CDCl_3$, ∂0: (t, 3H, -CH_3), 0.9 singlet, (Aryl-OH) 4.40

multiplet,(N-H),4.6 singlet, (Hetrocyclic amine) 7.30 singlet ; MS (m/z). 272 (100%, M^+)

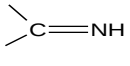
Synthesis of 7-Methyl-4-oxo-3, 4, 5, 6, 7, 8-hexahydro[1]benzothieno [2,3-d]pyrimidin-2-yl-acelic acid

IR (kBr, cm^{-1}): (C-CH_3 strech) 1390.84, (C=O) 1566.28,  1177.59, (thiophene) 954.50, () 1490.07, ^{1}HNMR ($CDCl_3$, ∂); (t, 3H, -CH_3) 1.09 doublet, (N-H) 4.5 Singlet, (Hetrocyclic amine) 7.23 Singlet, (Acids), 2.60 Multiplet; MS : (m/z) 278 (100% M^+)

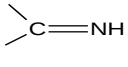
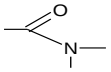
Synthesis of 6-(4-bromophenyl)-2, 5-dimethylthieno [2,3-d] pyrimidin-4(3H)

Ir(KBr,cm^{-1}): 3403.54 (), 1385.66(C-CH_3),3413.19(N-H stretch),1632.61(C=O),1608.09,(C-S),1174.70 (C-Br); ^{1}HNMR($CDCl_3$,∂); (R-C-Br).Sharp.3.11(C-CH_3),0.9 singlet,9.47 singlet(Aldehydic R-CH=O),7.21 multiplet (hetrocyclic amine); MS: m/z 335.21 (M^+,100%) % M^{+2} at 336 .

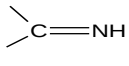
Synthesis of 2,5-dimethyl-6-(4-nitrophenyl) thieno[2, 3-d] pyrimidin-4(3H)-one

IR(KBr,cm^{-1}):() 1597.13,857.70(Thiophene),1568.12 (C=O), 1523.07 (C-NO_2), 2883.70 (-CH_3), ^{1}HNMR ($CDCl_3$,∂); (N-H) singlet 4.72, 9.27(aldehydic R-CH=O),7.31(hetrocyclic amine); MS; m/z 301.32 (M^+ ,100%) .

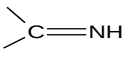
Synthesis of 2, 3-dihydroxy-3-(7-methyl-4-oxo-3, 4, 5, 6, 7, 8-hexahydro[1] benzothieno[2, 3-d]pyrimidin-2-yl) propanoic acid

IR(KBr, cm^{-1}) : 1566.27 (), 916.23 (thiophene) 1369.87 (C-CH_3), 1491.04 (C=N), 3289.99 () ^{1}HNMR ($CDCl_3$, ∂); 0.9 singlet (C-CH_3) ,4.74 oublet (N-H), 7.33 multiplet (Hetrocyclic amine), 9.31 Singlet (Aldehydic R-CH=O); MS : m/z, 324.35 (M^+,100%).

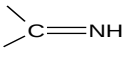
Synthesis of 5-Methyl-6-phenylthieno [2, 3-d]pyrimidin-4(3H)-one

IR(KBr, cm^{-1}) : 3415.20 (N-H strech),1392.08(C-CH_3) 1609.11(), 1663.65 (C=O), ^{1}HNMR ($CDCl_3$,∂); 0.9 singlet (-CH_3),4.71 singlet (N-H), 9.29 multiplet (Aldehydic R-CH=O), 7.42 triplet (Hetrocyclic amine),MS : m/z,249.29 (100% ,M^+).

Synthesis of 6-(2-hydroxyphenyl)-5-methylthieno[2, 3-d]pyrimidin-4(3H)-one

IR(KBr, cm^{-1}), 3420.18 (N-H strech),1387.18 (C-CH_3) 161.08 (), 1660.18 (C=O), ^{1}HNMR ($CDCl_3$,∂), 0.9 singlet (-CH_3), 4.69 singlet (N-H), 9.27 multiplet (Aldehydic R-CH=O), 7.27 triplet (Hetrocyclic amine, MS: m/z, 258.29, (M^+, 100%).

Synthesis of 6-(4-hydroxylphenyl)-5-methylthieno[2, 3-d]pyrimidin-4(3H)-one

IR(KBr, cm^{-1}), 3420.18 (N-H strech),1387.18 (C-CH_3) 161.08 (), 1660.18 (C=O), ^{1}HNMR ($CDCl_3$,∂), 0.9 singlet (-CH_3), 4.69 singlet (N-H), 9.27 multiplet (Aldehydic R-CH=O), 7.27 triplet (Hetrocyclic amine, MS: m/z, 258.29, (M^+, 100%).

Synthesis of 6-(4-aminophenyl)2,5-dimethylthieno[2,3-d] pyrimidin-4(3H)-one

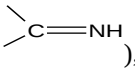
IR(KBr,cm^{-1}): 3423.20 (N-H strech), 1383.61 (C-CH_3), 1619.07 (),^{1}HNMR ($CDCl_3$, ∂) 4.9 doublet (R-NH-H) , 9.3 singlet (asldehydic R-CH=O), 7.31 doublet (Hetrocyclic amine) ; MS: m/z (271.83) (M^+, 100%) .

Table 1: Synthesized compound of thieno [2, 3-*d*]pyrimidin-4(3*H*)-one derivatives

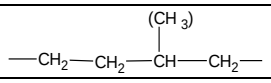
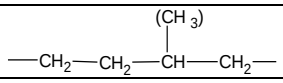
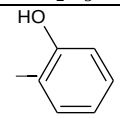
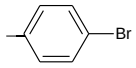
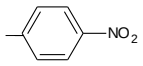
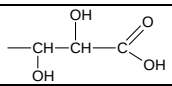
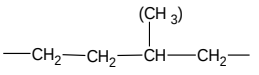
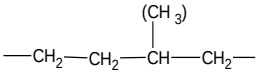
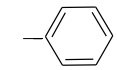
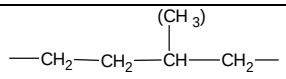
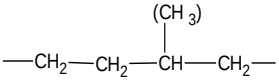
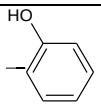
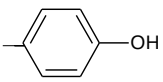
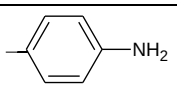
S. No.	Compound Code	R	R_1	R_2
1	2a	$-CH_3$		
2	2b	$-CH_3$	$-CH_3$	C_2H_5
3	2c	$-CH_3$	$-CH_3$	
4	2d	$-CH_3$	$-CH_3$	
5	2e	$-CH_3$	$-CH_3$	
6	2f			
7	2g	-H	$-CH_3$	
8	2h			
9	2i	-H	$-CH_3$	
10	2j	-H	$-CH_3$	
11	2k	$-CH_3$	$-CH_3$	

Table 2: Physicochemical properties of 2-amino-4, 5-substitutedthiophene-3-carbonitrile (1a-1h)

Comp. Code	Compound name	Molecular formula	Molecular weight (g)	% yield	R_f value	Melting point °C
1a	2-amino-6-methyl-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonitrile	$C_{10}H_{12}N_2S$	192.28	90.00	0.86	166-167
1b	5-ethyl-3-isocyano-4-methylthiophen-2-amine	$C_8H_{10}N_2S$	166.24	78.23	0.85	162-163
1c	2-(5-amino-4-isocyano-3-methylthiophen-2-yl)phenol	$C_2H_{10}N_2OS$	230.28	66.20	0.83	160-161
1d	5-(4-bromophenyl)-3-isocyano-4-methylthiophen-2-amine	$C_{12}H_9BrN_2S$	293.182	85.75	0.82	163-164
1e	3-isocyano-4-methyl-5-(4-nitrophenyl)thiophen-2-amine	$C_{12}H_9N_3O_2S$	259.284	78.67	0.87	157-158
1f	3-isocyano-4-methyl-5-phenylthiophen-2-amine	$C_{12}H_{10}N_2S$	214.28	87.35	0.93	155-156
1g	4-(5-amino-4-isocyano-3-methylthiophen-2-	$C_{12}H_{10}N_2OS$	230.28	77.80	0.81	161-162

	yl)phenol					
1h	5-(4-aminophenyl)-3-isocyano-4-methylthiophen-2-amine	$C_{12}H_{11}N_3S$	229.30	89.70	0.84	189-161

Table 3: Physicochemical properties of Thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives(2a-2k)

Comp Code	Compound Name	Molecular Formula	Molecular weight (g)	Melting Point °C	% Yield	R_f Value
2a	2,8 dimethyl-5,6,7,8-tetrahydrobenzo-[4,5]thieno[2,3-d]pyrimidin-4(3H)-one	$C_{12}H_{14}N_2OS$	234.31	203-204	86.00	0.83
2b	6-ethyl-2,5-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one	$C_{10}H_{12}N_2OS$	208.28	204-205	84.26	0.85
2c	6-(2-hydroxyphenyl)-2,5-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one	$C_{14}H_{12}N_2O_2$ S	272.34	206-207	82.75	0.90
2d	6-(4-bromophenyl)-2,5-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one	$C_{14}H_{11}BrN_2O$ S	335.21	209-210	80.33	0.93
2e)	2,5-dimethyl-6-(4-nitrophenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one	$C_{14}H_{11}N_3O_3S$	301.32	207-208	80.65	0.89
2f	2,3-dihydroxy-3-(7-methyl-4-oxo-3,4,5,6,7,8-hexahydro[1]benzothieno[2,3-*d]*pyrimidin-2-yl)propanoic acid	$C_{14}H_{16}N_2O_5S$	324.35	210-211	68.22	0.79
2g	5-methyl-6-phenylthieno-[2,3-d]pyrimidin-4(3H)-one	$C_{13}H_{10}N_2OS$	242.29	203-204	82.52	0.82
2h	7-methyl-4-oxo-3,4,5,6,7,8-hexahydro[1]benzothieno[2,3-*d]*pyrimidin-2-yl)acetic acid	$C_{13}H_{14}N_2O_3S$	278.32	198-199	66.15	0.83
2i	5-Methyl-6-phenyl thieno[2,3-d]pyrimidin-4(3H)-one	$C_{13}H_{10}N_2OS$	242.29	196-197	72.35	0.88
2j	6-(4-hydroxyphenyl)-5-methylthieno[2,3-d]pyrimidin-4(3H)-one	$C_{13}H_{10}N_2O_2S$	258.29	203-204	80.42	0.92
2k	6-(4-a minophenyl)-2,5-dimethylthieno[2,3-d]pyrimidin-4(3H)-one	$C_{14}H_{13}N_3OS$	271.33	209-209	83.00	0.79

Pharmacological Screening

Antimicrobial activity

The nutrient agar (Hi-media) medium was prepared dissolving 28g of nutrient agar in 1000ml of distilled water. The medium was sterilized by autoclaving at 15lb. pressure for 30 minutes. One loop full of the stock culture was inoculated at 10 ml of agar slant previously in sterilized test tube, and incubated at 37ºC for 24 hrs. for bacteria. About 3 ml of distilled water was added to the test tube and a suspension of the culture was obtained shaking for few minutes.

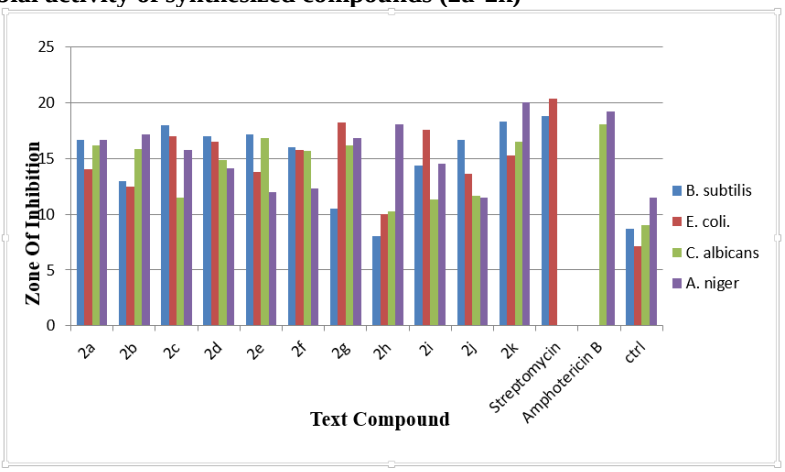
All the operation were carried out under aseptic conditions. Sterile medium was melted on water bath and kept at 45ºC in constant temperature water bath. In each sterile petri dish molten medium was added so that thickness was approximately 4-5 mm and sub cultured organisms under study was inoculated. The inoculated dishes were allowed to set for 30 min at room temperature. Cups of 6mm diameter were then made with the help of sterile stainless steel bore, a stock solution was added to bore in a concentration of 20, 50, 75 and 100µg/ml of each drug in each petri-plates. Petri

dishes were kept in refrigerator for 30 minutes so as to allow diffusion of the solution in the medium, and then incubated at 37ºC for 24 hrs. for antibacterial activity and 72 hrs. for antifungal activity. Zone of inhibition produced by test compounds were measure in mm and also minimum concentration of test drug required for inhibition and thus the compounds were selected on the basic of their effective concentration.

Table 4: Antimicrobial activity of synthesized compound (zone of inhibition* in mm, in 100µg/ml)

Compound code	Antibacterial Activity		Antifungal Activity	
	Gram (+)ve	Gram(-)ve	Fungi	
	B. subtilis	*E. coli.*	*C. albicans*	*A. niger*
2a	16.66±1.03	14.00±0.89	16.16±1.16	16.66±2.25
2b	13.00±0.89	12.5±1.04	15.83±1.83	17.16±0.75
2c	18.00±1.26	17.00±1.41	11.5±1.37	15.73±0.78
2d	17.01±0.97	16.5±3.01	14.83±0.75	14.16±2.31
2e	17.16±1.32	13.8±0.11	16.30±0.87	12.00±1.89
2f	16.00±1.09	15.8±1.32	15.66±2.06	12.33±0.81
2g	10.5±1.64	18.2±1.78	16.16±1.94	16.83±0.98
2h	8.00±2.19	10.00±1.54	10.27±0.91	16.00±1.67
2i	14.33±0.83	17.6±1.21	11.33±1.03	14.5±0.54
2j	15.33±1.36	13.6±1.21	11.66±1.03	11.5±1.22
2k	16.66±0.81	15.3±1.63	16.54±0.98	20.00±2.00
Streptomycin	23.83±1.32	21.33±1.86	-	-
Amphotericin B	-	-	18.03±1.67	19.25±1.50
Control	8.66±1.63	7.16±1.47	9.00±1.67	11.5±1.04

Graph 1: Antimicrobial activity of synthesized compounds (2a-2k)



The anti-microbial activity of synthesized derivatives was checked up by cup-plate dilution method. Microorganism is used primarily as an indication for compounds which are effective against bacteria and fungi. from **Table No.4** it was found that compounds **2a, 2c, 2d, 2e, 2f, 2g, 2j** and **2k** showed good whereas compounds **2b, 2h** and **2i** showed significant antibacterial activity compared with standard Streptomycin. And it also found that the compounds **2a, 2b, 2d, 2e, 2f, 2g, 2h** and **2k** showed good antifungal activity where as other i.e, **2c, 2i** and **2j** showed significant antifungal activity compared with standard Amphotericin B. All the synthesized compounds**(2a-2k)** gives better antimicrobial activity with **MIC value 100µg/ml** using bacterial strain *E. Coli* and *B. subtilis* and fungal strain *C. albicans* and *A. niger*.

Discussion

The thieno [2,3-*d*]pyrimidin-4(3*H*)-one derivatives were successfully prepared by new synthetic route and further purified and recrystalized by using ethanol as a solvent and purity was yet again checked by thin layer chromatographic technique.

The title compound was further characterized by physicochemical method and spectral analysis. Melting point was recorded by two different method capillary tube method and visible melting point apparatus method and was uncorrected. TLC was done to determine purity by using solvent toluene: ethyle acetate (7:3) and R_f value was reported.

The Infrared spectra for the synthesized compounds were recorded using SHIMADZU - FTIR IRA – Affinity 1 and absorbance peaks were recorded using KBr pellets. This is further supported by NMR studies and Mass studies.

The actual IR, NMR and Mass spectra of the synthesized compounds are given in above figures. The interpretation was carried out by observing the graph.

FTIR spectra of all compound showed aromatic C=O stretching vibration 1665.60 cm^{-1}. All derivatives showed a broad absorbance band at about 1490-1580 cm^{-1} associated with stretching vibrations of bonded N-H, indicating present of nitrogen. Each compound showed a strong absorbance due to presence of C-S at 980-1225 cm^{-1}. All derivatives showed broad absorbance at about 1640-1690 cm^{-1} associated with stretching vibrations of bonded –N=C-, indicating present of nitrogen in the ring. Compound 2d showed a strong absorbance at 1350-1560 cm^{-1} stretching vibration indicating present of C-NO_2 group. Compounds 2e showed absorbance at 1030-1075 cm^{-1} stretching vibration indicating present of Br group.

The structures of synthesized compounds are further confirming by NMR and Mass spectra. ^{1}HNMR of compounds 2b, 2c and 2h shows a sharp singlet peak at 7-7.5 ppm, indicating presence of heteroaromatic amine and also Singlet to multiplet peak at 4-4.7 ppm, indicating presence of s 1H-N. The compound 2b and 2h shows sharp singlet peak at 9-10 ppm, indicating the presence of Aldehydic R-CH=O. The broad multiplet peak in compound 2c at 4.40 ppm, indicating the presence of Aryl-OH. The compound 2b and 2c shows sharp singlet to doublet peak indicating the presence of primary proton at 0.9 ppm and compound 2c shows broad multiplet peak at 1.31 ppm, indicating the presence of secondary proton. The Mass spectra of compound 2b, 2c and 2h shows M+ peak at 208, 272 and 278 respectively.

Conclusion

All the newly synthesized compounds were screened in vitro for their preliminary antimicrobial testing of compound 2a-2k. The result showed that the entire of synthesized compounds exhibit good, moderate and weak antimicrobial activity as compared with standard. It was observed that the compounds 2a, 2c, 2d 2e, 2f, 2g, 2j and 2k showed good antimicrobial activity whereas compound 2b, 2i and 2j showed significant antimicrobial activity on bacterial strain that is *E. coli* and *B. subtilis* and fungi strain that is *C. albicans* and *A. niger* when compared with standard Streptomycin and Amphotericin B respectively.

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