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Cytotoxicity Analysis of Some Novel Pyrimidine Derivatives

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ABSTRACT

Some pyrimidine derivatives are synthesized by one pot multicomponent condensation. All the compounds are characterized by FT-IR, NMR analysis. Anti-microbial activities of the synthesized compounds are evaluated by disc diffusion method, and results show that some of the compounds show good activity and the residual compounds show moderate activity. Toxicity of the compounds has been analysed by MTT assay using colo320 cell line.

Keywords: Pyrimidine; Multivariate curve resolution; Nuclear magnetic resonance; MTT assay

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INTRODUCTION

The present attractiveness of pyrimidine core is mainly due to their valuable pharmacological properties. A number of synthetic pyrimidine and fused pyrimidine pharmacophores exhibit antibacterial, antiviral, antimicrobial, anticancer, cytotoxic, antioxidant, antihypertensive, cardiac stimulant, antimalarial, anti-HBV, anti-HIV-1, and antirubella virus activities [1-10]. In that point of view, multi component reaction is one of the best methods to synthesis sustainable organic compounds and to developing collections of medicinal scaffolds [11-22].
Due to long lasting interest in pyrimidine and fused pyrimidine derivatives as potential rings, in this frame work, we planned to synthesis some pyrimidine derivatives by one pot Biginelli condensation method and their microbial activity are evaluated against bacterial and fungal pathogens, Also cytotoxicity of the compounds were evaluated against human colo320 cell lines.

MATERIALS AND METHODS

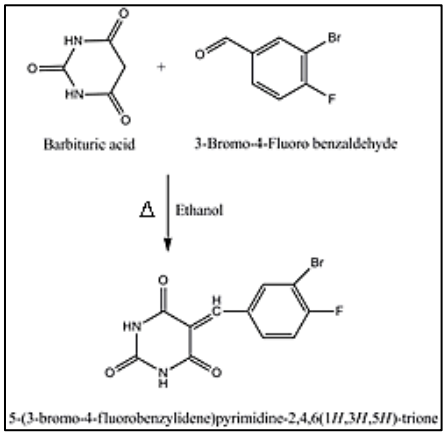
All the chemicals used in this study were purchased from sigma Aldrich and Merck chemical company. The melting point of the synthesized compounds have been determined in open capillaries using mettle FP51 apparatus expressed in °C and are uncorrected. Infrared spectra (KBr, 4000-400 cm^{-1}) of all the synthesized compounds have been measured by using SHIMADZU Fourier transform spectrophotometer at Centre of advanced marine biology, Annamalai University, Parangipettai and the FT-Raman spectrum was recorded using Bruker RFS 27 spectrometer operating at laser 100 mW in the spectral range 3500-50 cm^{-1} at Indian Institute of Technology, Chennai. NMR spectra recorded using Bruker spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR spectrum, with DMSO-d^{6}/$CDCl_3$ as solvent at Annamalai University, Chidambaram. The chemical shift values are recorded in ppm relative to TMS.

Chemistry

From the literature, Fused pyrimidine derivatives have more biological potential [23-25], in order to ensure that, we have synthesize four fused pyrimidine derivative and one substituted pyrimidine derivative by microbial evaluation and cytotoxicity assay. The synthetic procedures are given below.

Synthesis of Pyrimidine Derivative

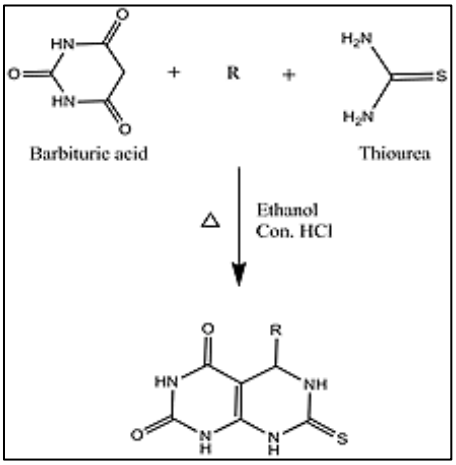
Equal mole ratio (0.0098 mol) of barbituric acid and 3-Bromo-4-fluourobenzaldehyde are dissolved in ethanol and the content is condensed on oil for bath for 1 hour then the reaction mixture is poured in water. The product obtained is filtered and is recrystallized from ethanol. The completion of the reaction was monitored using thin layer chromatography technique.



Scheme 1: Synthesis of pyrimidine derivative (1)

Synthesis of Fused Pyrimidine Derivative

Tetra hydro pyrimidines are synthesised by multicomponent condensation of mixture of barbituric acid, thiourea and various aldehydes are dissolved in ethanol. To this mixture, 5 ml of conc. HCl is added then the content is condensed on oil bath for 1 hour then the reaction mixture is poured in water and the product obtained are filtered and dried (Schemes 1 and 2).



Scheme 2: Synthesis of fused pyrimidine derivative (2-5)

Where, R = (2) 4-Hydroxy-3-methoxy benzaldehyde
(3) 4-Difluoromethoxy-3-Hydroxy benzaldehyde
(4) Acetaldehyde
(5) 3, 5-Difluoro-4-hydroxybenzaldehyde

The synthesized compounds are characterized by FT-IR, FT-Raman, NMR spectral analysis and the representative spectrums are shown in Figures 1-20 (S1-S20).

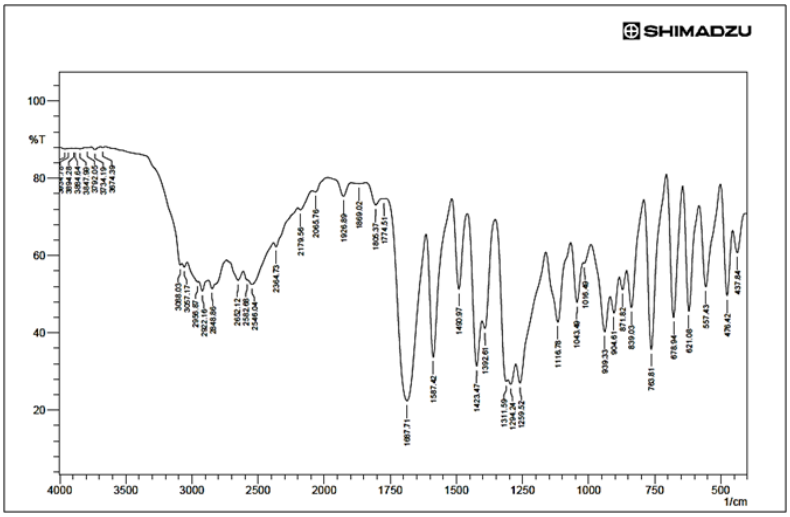


Figure 1: The FT-IR spectrum of compound 1 (S1)

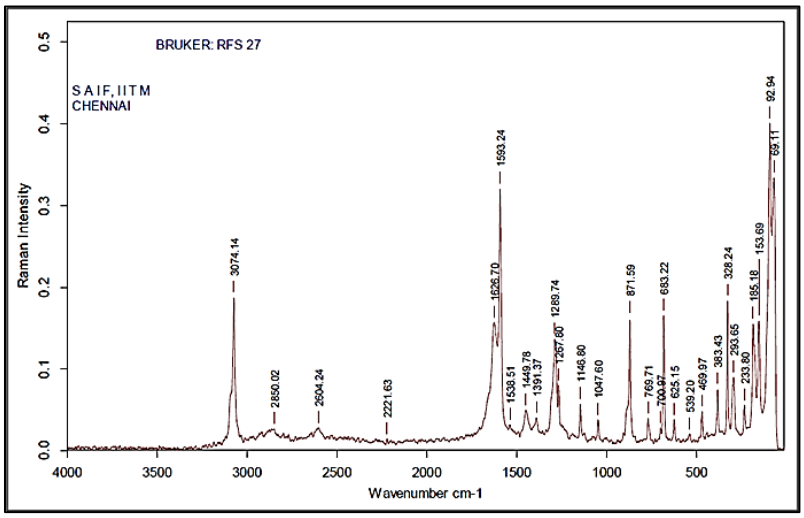


Figure 2: The FT-Raman spectrum of compound 1(S2)

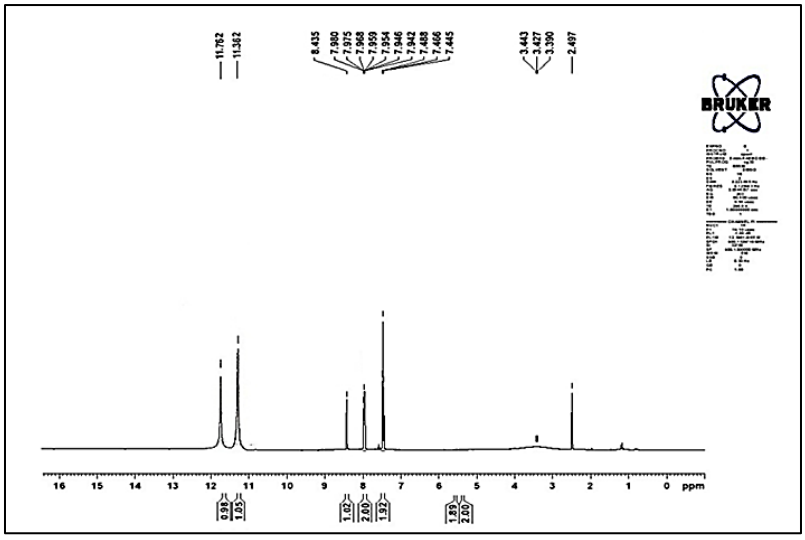


Figure 3: The 1H NMR spectrum of compound 1 (S3)

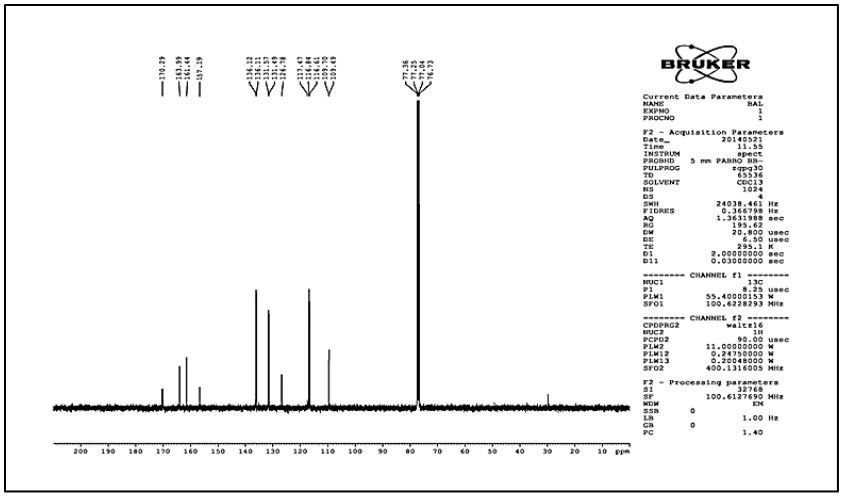


Figure 4: The 13C NMR spectrum of compound 1 (S4)

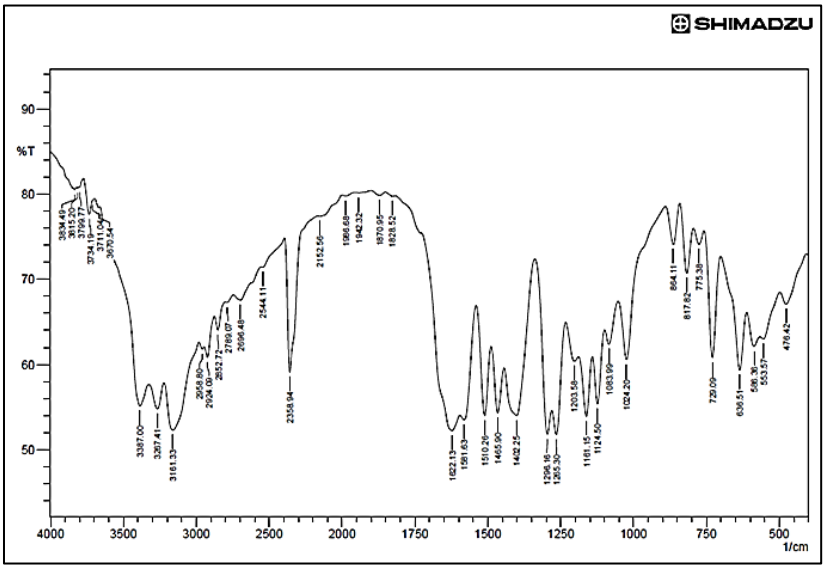


Figure 5: The FT-IR spectrum of compound 2 (S5)

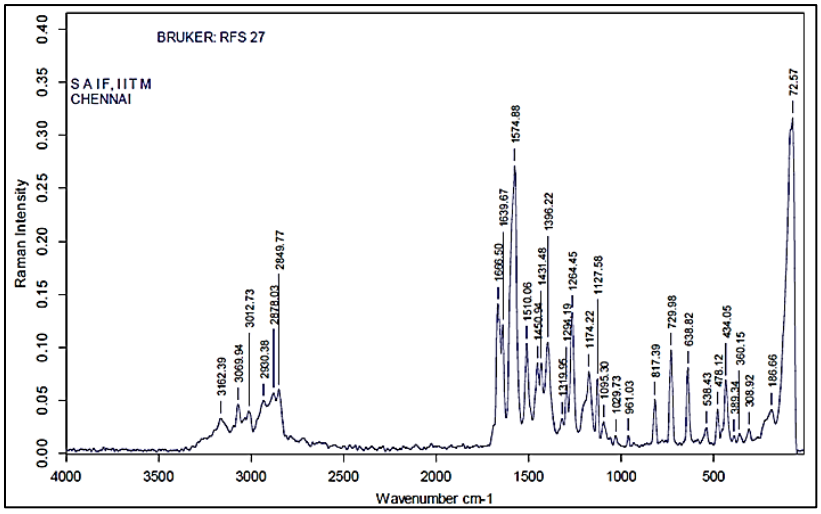


Figure 6: The FT-Raman spectrum of compound 2 (S6)

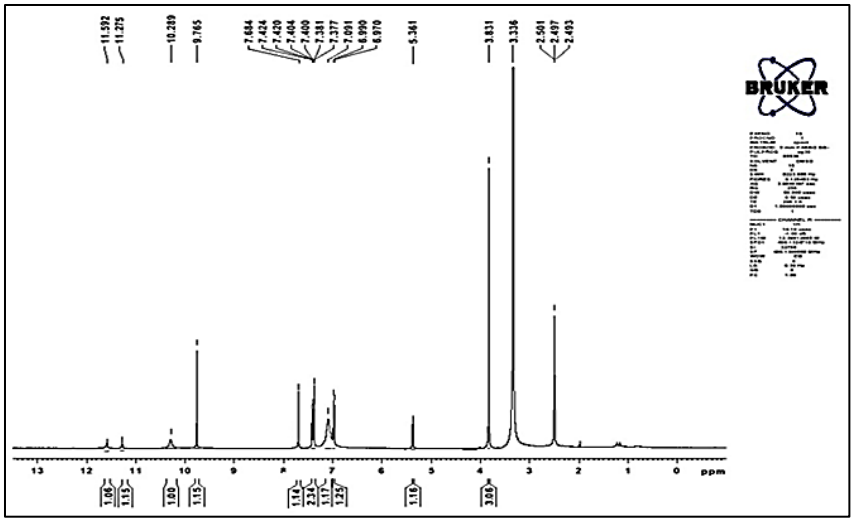


Figure 7: The 1H NMR spectrum of compound 2 (S7)

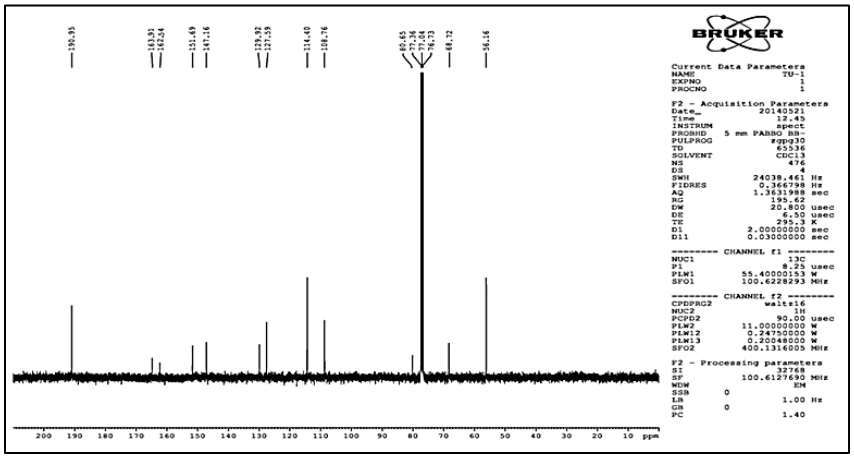


Figure 8: The 13C NMR spectrum of compound 2 (S8)

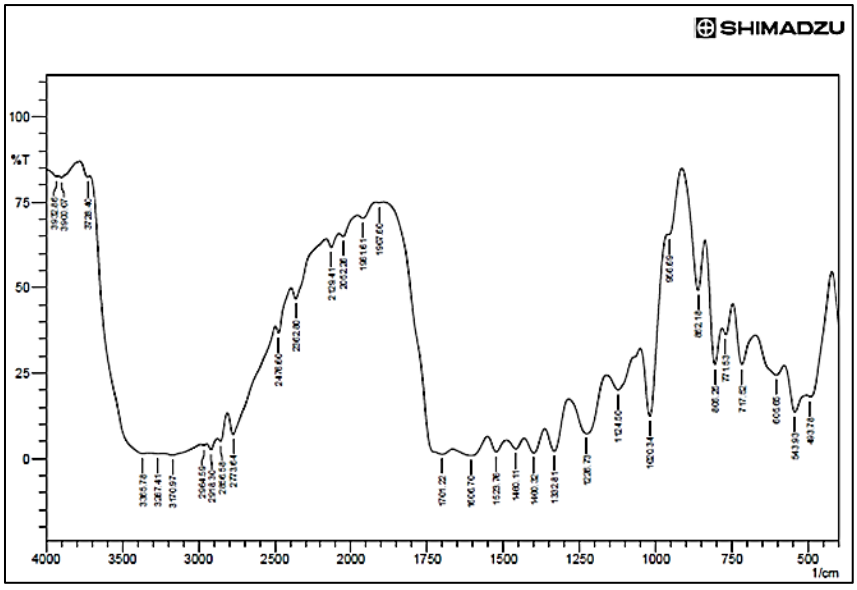


Figure 9: The FT-IR spectrum of compound 3 (S9)

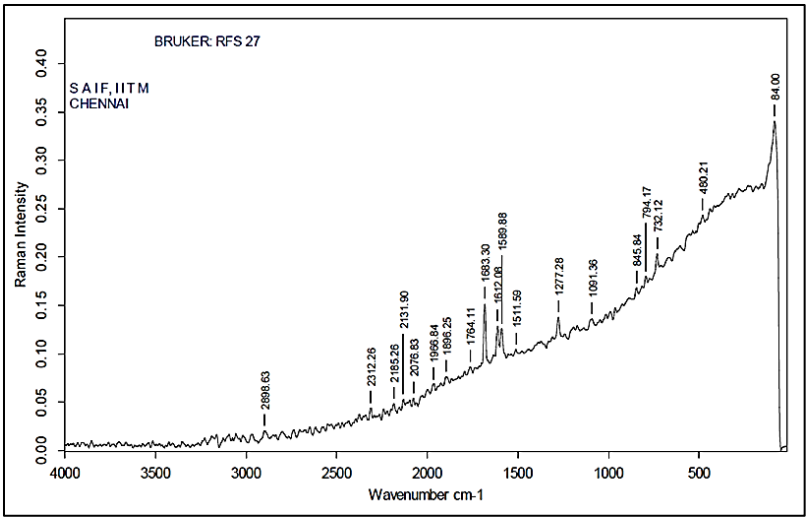


Figure 10: The FT-Raman spectrum of compound 3 (S10)

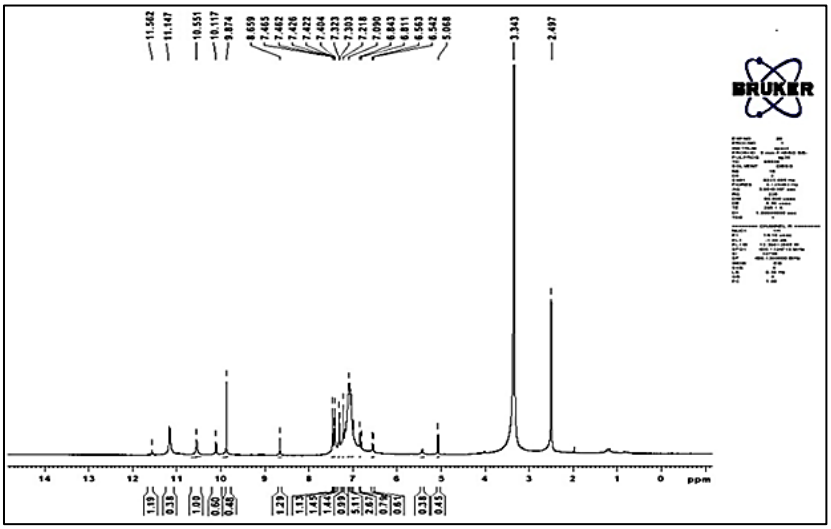


Figure 11: The 1H NMR spectrum of compound 3 (S11)

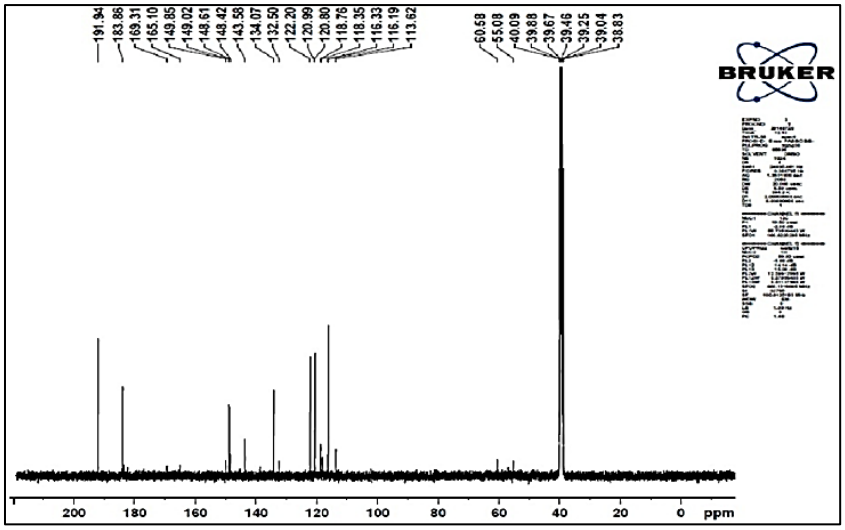


Figure 12: The 13C NMR spectrum of compound 3 (S12)

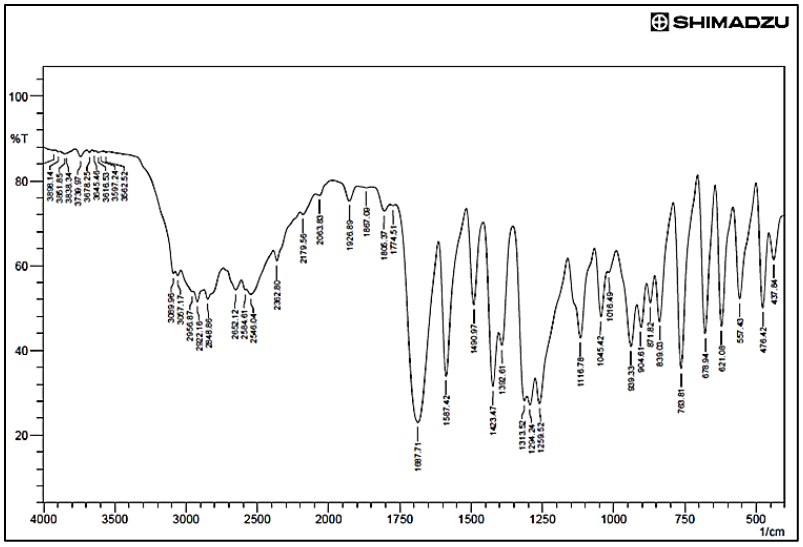


Figure 13: The FT-IR spectrum of compound 4 (S13)

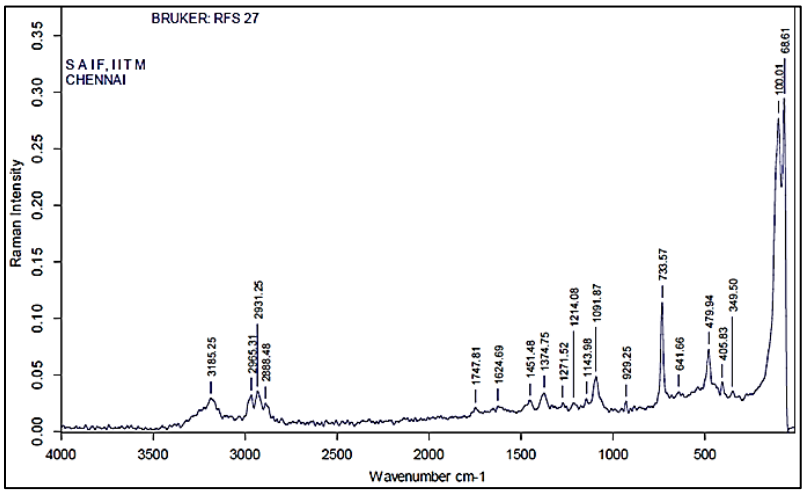


Figure 14: The FT-Raman spectrum of compound 4 (S14)

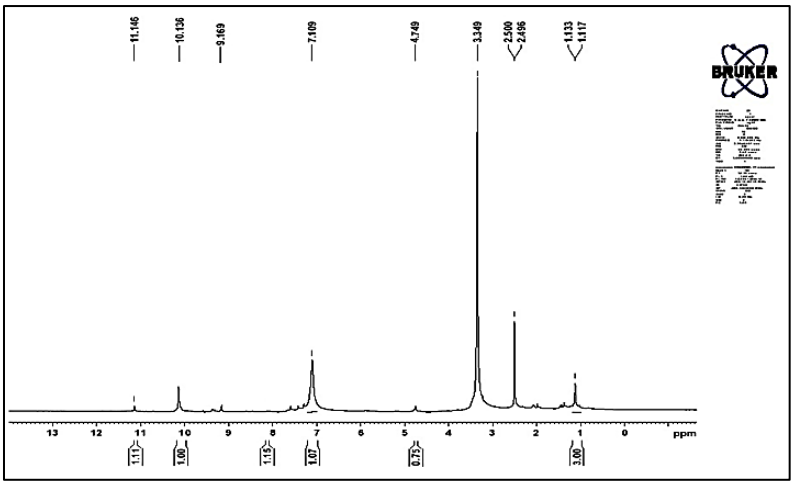


Figure 15: The 1H NMR spectrum of compound 4 (S15)

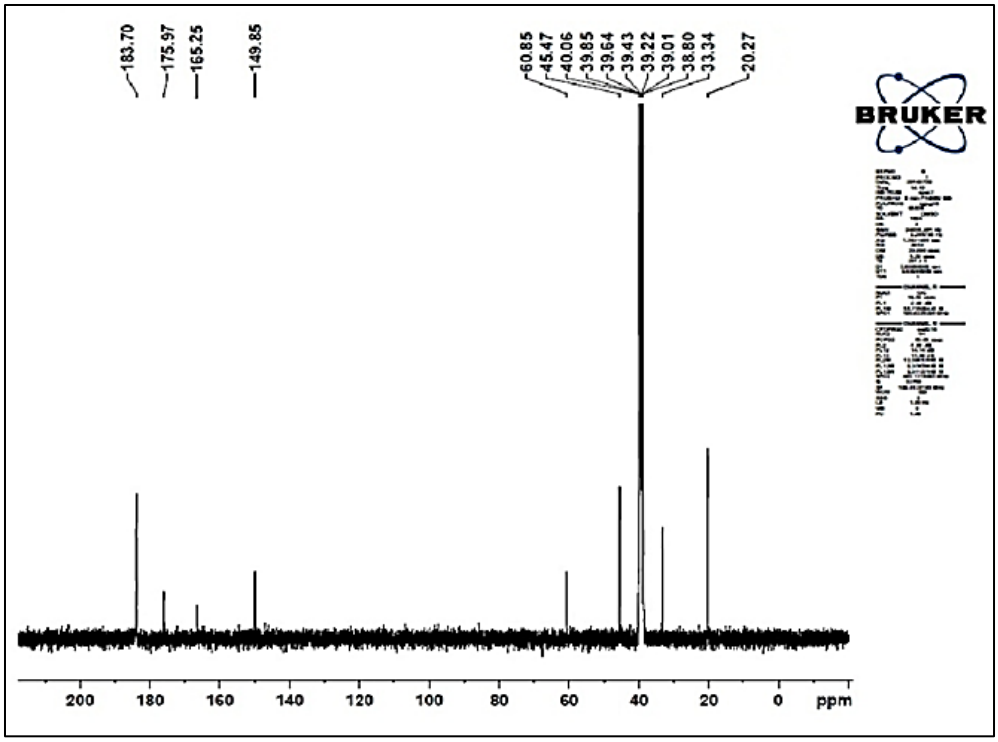


Figure 16: The 13C NMR spectrum of compound 4 (S16)

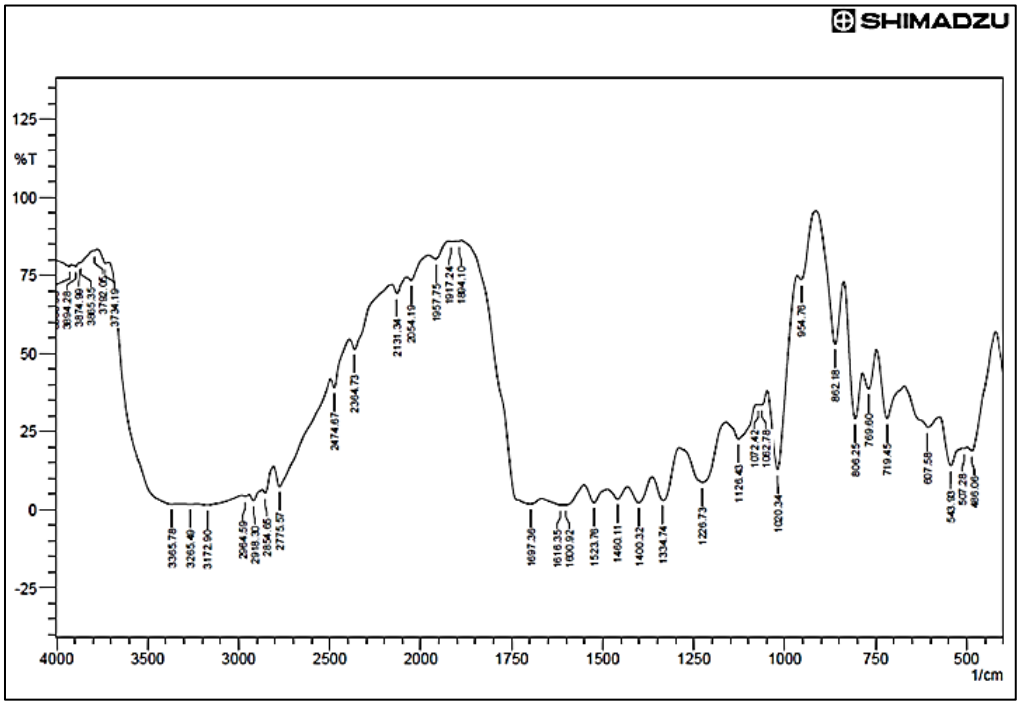


Figure 17: The FT-IR spectrum of compound 5 (S17)

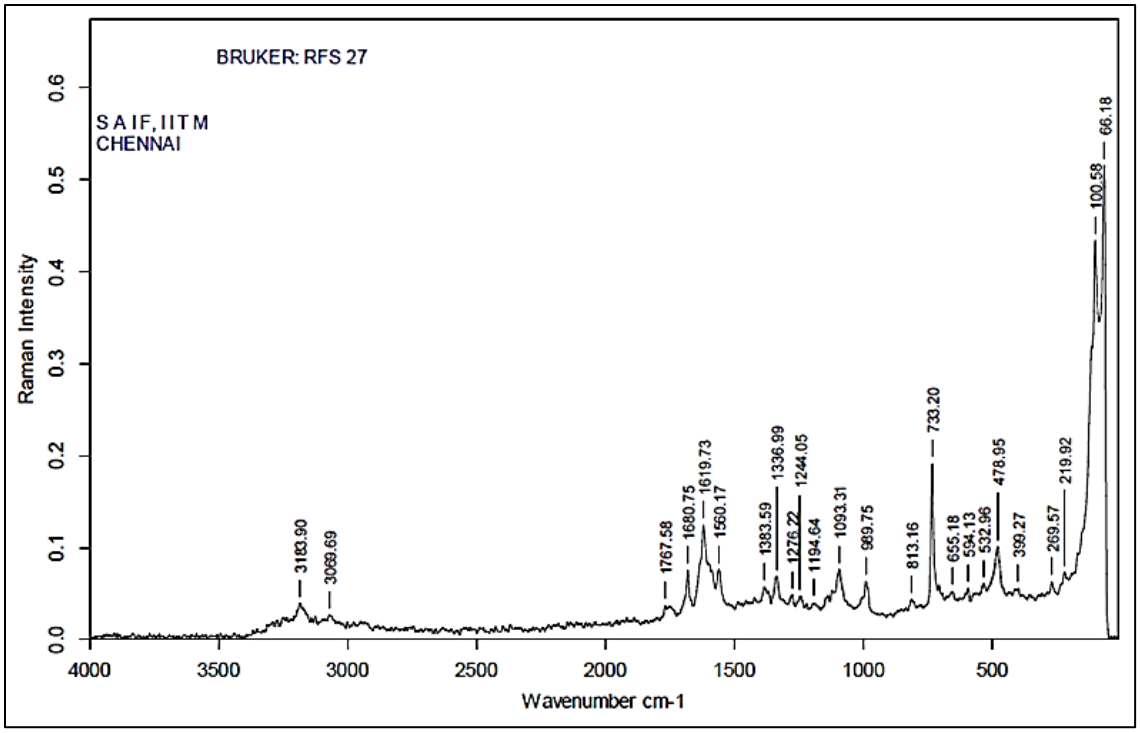


Figure 18: The FT-Raman spectrum of compound 5 (S18)

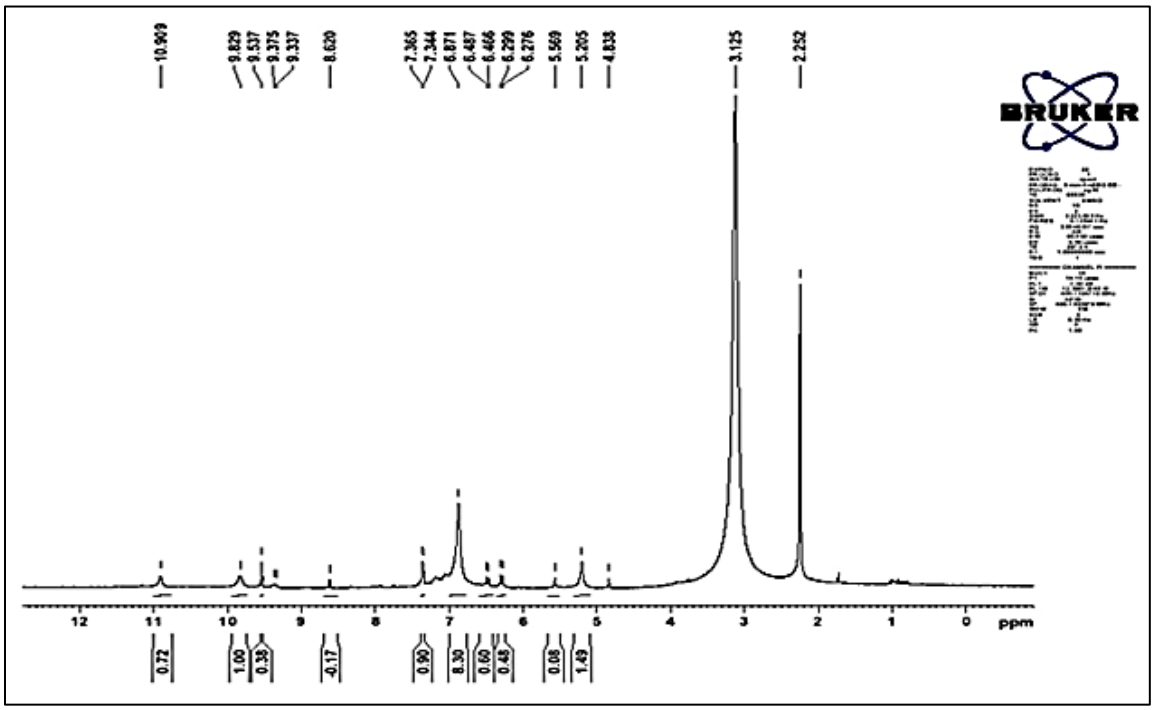


Figure 19: The 1H NMR spectrum of compound 5 (S19)

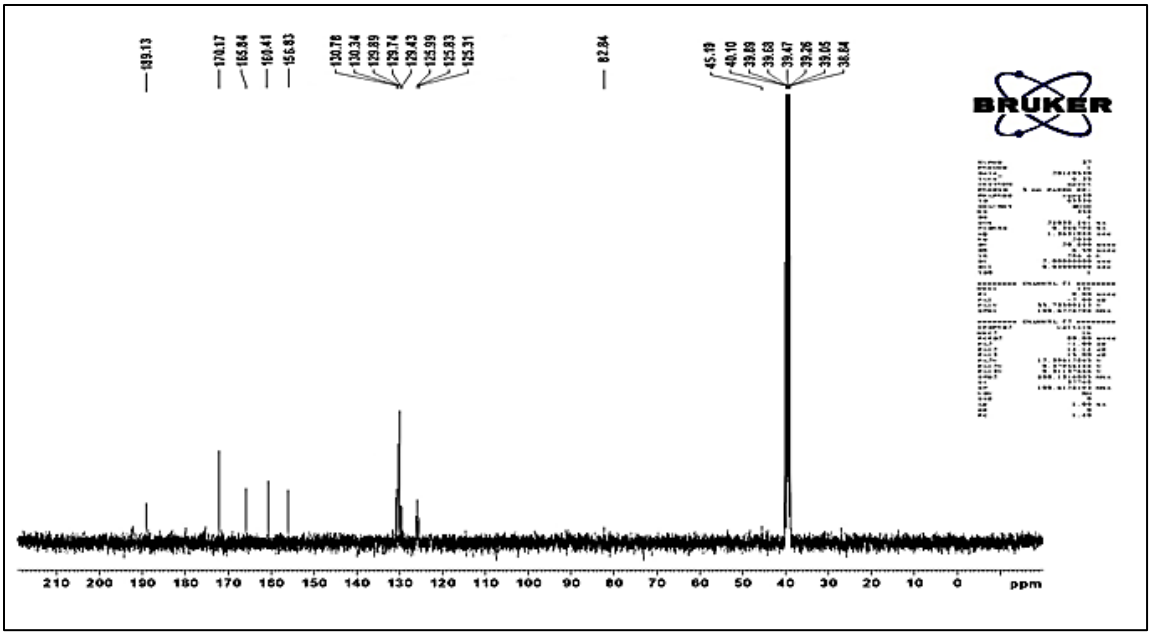


Figure 20: The 13C NMR spectrum of compound 5 (S20)

5-(3-bromo-4-fluorobenzylidene) pyrimidine-2,4,6(1H,3H,5H)-trione (1):
IR (KBr) ν cm^{-1}: 3444, 3221 (NH), 3115 (Aromatic C–H), 1691 (C=O), 1593 (C=C); Raman (ν cm^{-1}): 3734 (OH), 3183 (NH), 3069 (Aromatic), 2878 (CH3), 1680, 1619 (C=O), 1523 (C=C), 1093 (C=S); ^{1}H-NMR (400 MHz**,** $DMSOd_6$): δ = 7.48-7.44 (m, 1H), 7.98-7.95 (m,1H), 8.15-8.13 (m,1H), 9.0 (s,1H, =CH).;^{13}C-NMR (100 MHz, $DMSOd_6$): δ= 109.5, 116.7, 117.47, 126.78, 131.5, 136.1, 161.4, 163.9, 170.2.

5-(4-hydroxy-3-methoxyphenyl)-7-thioxo-5,6,7,8-tetrahydropyrimido(4,5-d)pyrimidine-2,4(1H,3H)-dione: (2)
IR (KBr) νcm^{-1}:3670(OH), 3387 (NH), 2958 (Aromatic), 2924(Ar-CH), 2852 (CH3), 1622 (C=O), 1581 (C=C), 1024 (C=S); Raman (ν cm^{-1}): 3162 (NH), 3012 (Aromatic), 2930 (Ar-CH), 2878 (CH3), 1666, 1639 (C=O), 1574 (C=C), 1029 (C=S); ^{1}H-NMR (400 MHz, $DMSOd_6$): 5.36 (s, 1H, CH), 6.97-7.68 (m, 3H, ArH), 9.76 (s, 1H, NH), 10.28(s, 1H, NH), 11.27(s, 1H, NH), 11.59 (s, 1H, NH). 7.09 (s, 1H, OH), 3.83 (s, 1H, 0CH3); ^{13}C-NMR (100 MHz, $DMSOd_6$): 162.54, 163.91, 190.95, 108.76-151.61, 80.65, 68.72, 56.16.

5-(4-(difluoromethoxy)-3-hydroxyphenyl)-7-thioxo-5,6,7,8-tetrahydropyrimido(4,5-d)pyrimidine-2,4(1H,3H)-dione: (3)
IR (KBr) νcm^{-1}: 3728 (OH), 3365 (NH), 2964 (Aromatic), 2918(Ar-CH), 2856 (CH3), 1701, 1606 (C=O), 1523 (C=C), 1020 (C=S); Raman (ν cm^{-1}): 3162 (NH), 3012 (Aromatic), 2930 (Ar-CH), 2878 (CH3), 1666, 1639 (C=O), 1574 (C=C), 1029 (C=S); ^{1}H-NMR (400 MHz, $DMSOd_6$): 5.08 (s, 1H, CH), 6.54-7.46 (m,ArH), 10.11 (s, 1H, NH), 10.55 (s, 1H, NH), 11.14 (s, 1H, NH), 11.56 (s, 1H, NH), 9.87 (s, 1H, OH); ^{13}C-NMR (100 MHz, $DMSOd_6$):165.10, 169.31, 191.94, 183.86, 113.62-149.85, 55.08, 60.58.

5-methyl-7-thioxo-5,6,7,8-tetrahydropyrimido(4,5-d)pyrimidine-2,4(1H,3H)-dione: (4)
IR (KBr) νcm^{-1}: 3089 (NH), 2922(C-CH), 2956 (CH3), 1774, 1687 (C=O), 1587 (C=C), 1045 (C=S); Raman (ν cm^{-1}): 3185 (NH), 2931 (C-CH), 2956 (CH3), 1747, 1624 (C=O), 1091 (C=S); ^{1}H-NMR (400 MHz, $DMSOd_6$): 4.75 (s, 1H, CH), 7.19 (s, 1H, NH), 9.16 (s, 1H, NH), 10.13 (s, 1H, NH), 11.14(s, 1H, NH), 1.13 (s, 1H, CH3);^{13}C-NMR (100 MHz, $DMSOd_6$):165.25, 175.97, 183.70, 60.85, 45.47, 20.27.

5-(3,5-difluoro-4-hydroxyphenyl)-7-thioxo-5,6,7,8-tetrahydropyrimido(4,5-d)pyrimidine-2,4(1H,3H)-dione (5)
IR (KBr) ν cm^{-1}: 3734(OH), 3365 (NH), 2964 (Aromatic), 2918(Ar-CH), 1697, 1616 (C=O), 1523 (C=C), 1062 (C=S); Raman (ν cm^{-1}): 3734 (OH), 3183 (NH), 3069 (Aromatic), 2878 (CH3), 1680, 1619 (C=O), 1523 (C=C), 1093 (C=S); ^{1}H-NMR (400 MHz, $DMSOd_6$): 5.20 (s, 1H, ArCH), 6.27-7.36 (m, ArH), 9.37 (s, 1H, NH), 9.53 (s, 1H, NH), 9.83 (s, 1H, NH), 10.91 (s, 1H, NH). 5.56 (s, 1H, OH); ^{13}C-NMR (100 MHz, $DMSOd_6$):189.13, 165.84, 170.17, 125.31-160.41, 82.84, 45.19.

Procedures for Biological Evaluation

Anti-microbial evaluation:

The anti-microbial activities of the synthesized compounds are evaluated against each five pathogenic bacterial and fungal species. Antimicrobial sensitivity assay was performed by using Kirby-Bauer disc diffusion technique [26].

Antibacterial Activity

Antibacterial activity of the synthesized compounds was determined against Gram-positive bacteria in DMF by disc diffusion method on nutrient agar medium. The sterile medium (Nutrient Agar Medium, 15 ml) in each petri-plates was uniformly smeared with cultures of Gram positive and Gram negative bacteria. Sterile discs of 10 mm diameter (Hi-Media) was placed in the petri-plates, to which 50 µl (1 mg/ml i.e., 50 µg/disc) of the synthesized compounds were added. The treatments also included 50 µl of DMF as negative, DMSO as positive control for comparison. The plates were incubated at 37 ± 2°C for 24 h and the zone of inhibition was determined in mm and ciprofloxacin used as a standard.

Antifungal Activity

The synthesized compounds were screened for their antifungal activity against *Fusarium oxysporum, Aspergillus flavus, Aspergillus niger, Penicillium chrysogenum and Trigoderma veride* in DMF. Potato Dextrose Agar (PDA) media was prepared and about 15 ml of PDA was poured into each Petri plate and allowed to solidify. 5 mm disc of seven days old culture of the test fungi was placed at the centre of the petri plates and incubated at 26°C for 7 days. After incubation the percentage inhibition was measured. Amphotericin-B was used as standard.

Cytotoxicity Analysis of Pyrimidine Derivatives

Procedure:

The MTT colorimetric assay has been performed to analyse the cytotoxicity of the synthesized compounds. Colo320 cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37°C in humidified atmosphere with 5% CO_2. Cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2×10^4 cells/well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated with different concentrations of the extract for 24 hours. After the incubation, medium was discarded and 100µl fresh medium was added with 10µl of MTT (5 mg/ml). After 4 hours, the medium was discarded and 100 µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570 nm in a microtitre plate reader. The assay was performed for all 10 fractions obtained from column chromatography.

Cell survival was calculated by the following formula:

Viability % = (Test OD/ Control OD) x 100

Cytotoxicity % = 100 – Viability%

RESULTS AND DISCUSSION

Anti-bacterial Activity

In order to evaluate the anti-bacterial activity of the synthesized compounds, they were tested *in vitro* anti-bacterial screening against various grams positive and gram negative pathogenic bacteria stains and the zone inhibitions were compared with the standard ciprofloxacin and the results are presented in Table 1 and zone of inhibition shown in Figure 21. All the compounds show good to moderate activity against bacterial strains except compound 2, this compound show moderate inhibitory activity against *Pseudomonas aeruginosa.*

Anti-fungal Activity

In vitro antifungal activity of the synthesized compounds 1-5 was tested against (*Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Penicillium chrysogenum, Trigoderma veride).* The results were compared with the standard Amphotericine B and the results are depicted in the Table 2 and zone of inhibition shown in Figure 22.

Aspergillus flavus

The compound 1 shows good activity against *Aspergillus flavus* and other compounds show moderate activity.

Aspergillus niger

The excellent inhibition by compound 4 against *Aspergillus niger,* could be attributed to the presence of methyl group in the compound. The residual compounds show good activity except compound 5.

Fusarium oxysporum
On testing against *Fusarium oxysporum*, compound 3 show good activity and compound 1,4 and 5 shows moderate activity, while compound 4 show no activity against respective fungal species.

Penicillium chrysogenum
Compound 1 show good activity while other compounds show moderate activity.

Trigoderma Veride
All the compounds show good activity against fugal species; whereas compound 3 show no activity.

MTT Assay
In this study, in order to evaluate the cytotoxicity, the synthesized compounds are subjected to MTT assay. The colo360 cell is exposed to different concentration of test compounds. From the results, it is clear that compound 4 shows lower $IC5_0$ value compared to other compounds. The percentage of viability, toxicity and IC_{50} value of the compounds listed in the Table 3.
From the above discussions of microbial evaluation and MTT assay, it is clearly noted that the fused pyrimidines (2-5) show better activity then pyrimidine derivative (5), it is also evident from cytotoxicity analysis, IC_{50} value of compound 1 is greater than other compounds.

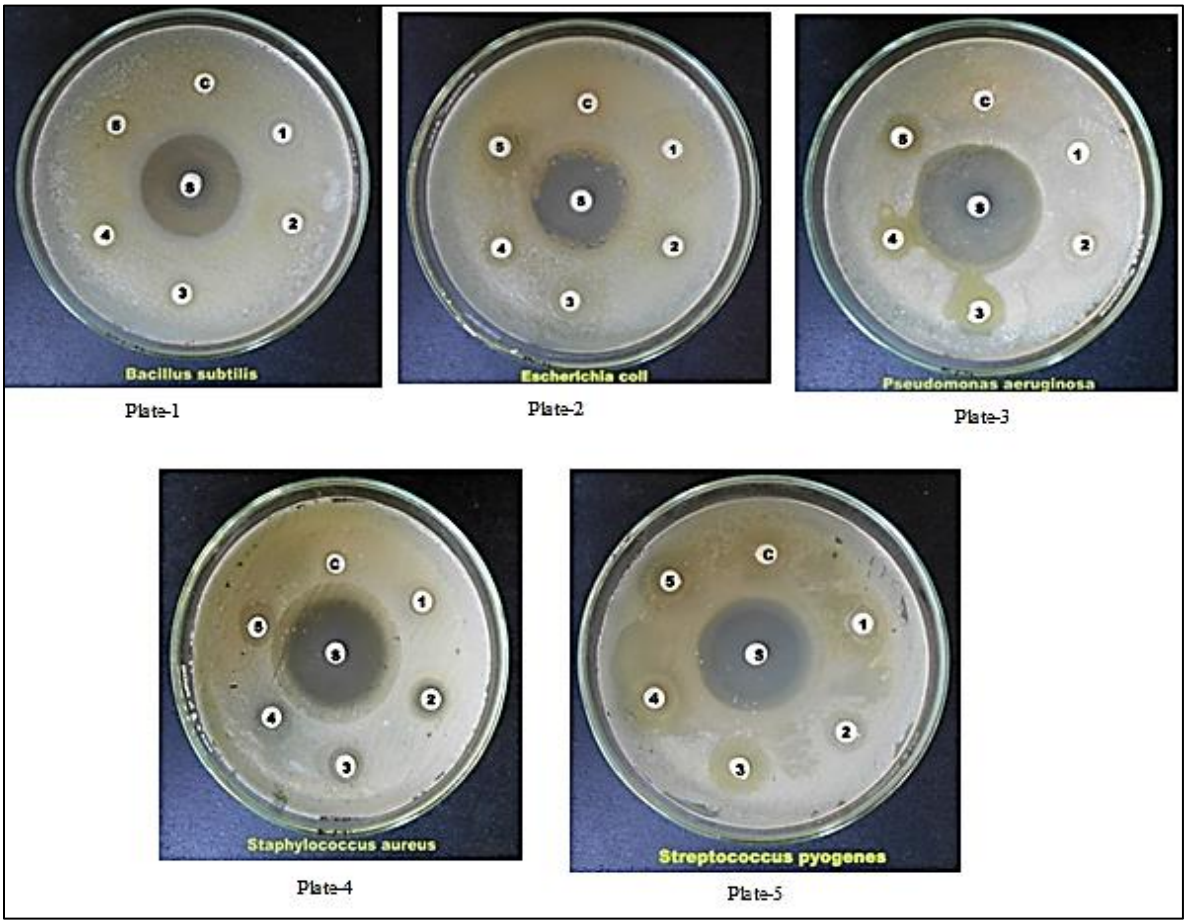


Figure 21: Anti-bacterial activity of pyrimidine derivatives

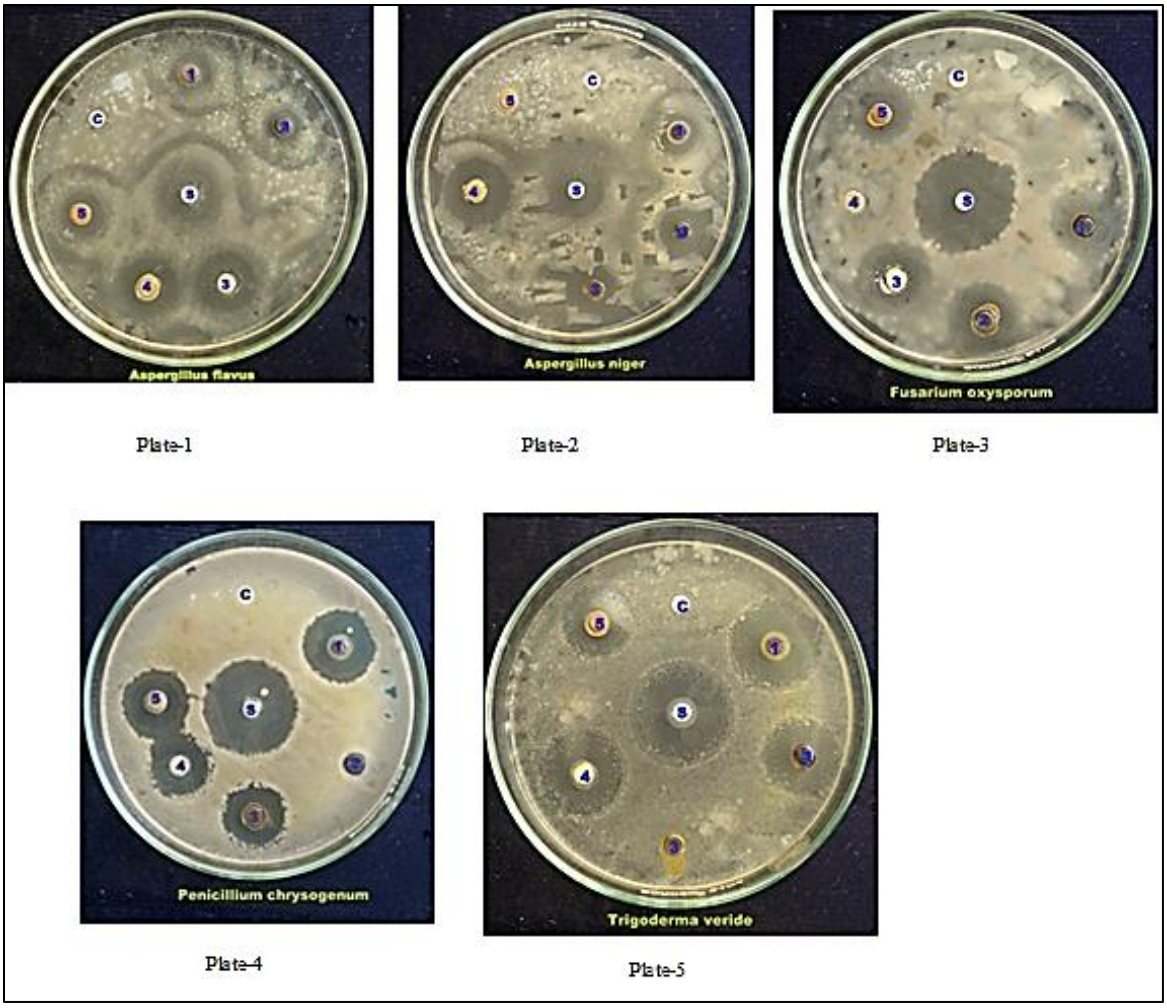


Figure 22: Anti-fungal activity of pyrimidine derivative

Table 1: Antibacterial activity of pyrimidine derivatives (disc diffusion method)

S. No.	Bacteria	Standard Antibiotic Disk*	Zone of inhibition (mm)				
			1	2	3	4	5
1	*Bacillus subtilis*	30	13	12	12	16	14
2	*Escherichia coli*	31	10	13	15	15	17
3	*Pseudomonas aeruginosa*	32	10	9	17	14	14
4	*Staphylococcus aureus*	22	10	14	14	16	17
5	*Streptococcus pyogenes*	32	12	15	20	16	16

Standard: ***ciprofloxacin**

Table 2: Antifungal activity of pyrimidine derivatives (Disc diffusion method)

S. No.	Bacteria	Standard Antibiotic Disk*	Zone of inhibition (mm)				
			1	2	3	4	5
1	*Aspergillus flavus*	28	21	19	17	17	14
2	*Aspergillus niger*	18	14	15	16	21	-
3	*Fusarium oxysporum*	22	16	16	18	-	16
4	*Penicillium chrysogenum*	19	17	-	13	12	13
5	*Trigoderma veride*	24	22	20	-	21	19

Standard: Amphotericin-B

Table 3: Cytotoxicity analysis of pyrimidine derivatives

Sample	Concentration	Control	Sample	% of viability	% of toxicity	IC50 value
	Control	1.121	1.121	100	0	
1	50 µg	1.121	0.961	85.727	14.2729	186.03
	100 µg	1.121	0.845	75.3791	24.6208	
	150 µg	1.121	0.766	68.3318	31.6681	
2	50 µg	1.121	0.984	87.7787	12.2212	171.24
	100 µg	1.121	0.822	73.3273	26.6726	
	150 µg	1.121	0.713	63.6039	36.396	
3	50 µg	1.121	0.921	82.1587	17.8412	168.19
	100 µg	1.121	0.812	72.4353	27.5646	
	150 µg	1.121	0.695	61.9982	38.0017	
4	50 µg	1.121	0.841	75.0223	24.9776	151.73
	100 µg	1.121	0.712	63.5147	36.4852	
	150 µg	1.121	0.652	58.1623	41.8376	
5	50 µg	1.121	0.935	83.4076	16.5923	165.53
	100 µg	1.121	0.812	72.4353	27.56467	
	150 µg	1.121	0.684	61.0169	38.983	

CONCLUSION

In this frame work, five pyrimidine derivatives were synthesized. The structures of the compounds are determined by FT-IR, FT-Raman, NMR spectral analysis. The microbial activity of the compounds are evaluated by disc diffusion method, the results shows that some of the compounds show moderate to good activity against bacterial and fungal stains, in which the methyl group substituted fused pyrimidine compound (4) show excellent activity against *aspergillus niger* (fungal species) then standard Amphotericin-B. In order to evaluate the cytotoxicity of the compound MTT Assay has been performed, results shows that compound 4 show IC_{50}=151.73, which is low compared to other compound. From these evaluation, compound 4 show excellent activity with low IC50 value hence this compound may act as a drug with further modification.

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