International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar – Apr 2025, pp: 421-427 www.ijprajournal.com ISSN: 2456-4494

Synthesis of Dihydropyrimidine Derivative and Its Anti-Bacterial Activity

Author: K. Sumathi[1], Co-Author: S. Rajesh Kumar[2], N.Senthil Kumar[3], A.Anagha[4]

Associate Professor Department Of Pharmaceutical Chemistry, Annai J.K.K Sampoorani Ammal College Of Pharmacy, The Tamilnadu Dr.M.G.R. Medical University.
Student Department Of Pharmaceutical Chemistry, Annai J.K.K Sampoorani Ammal College Of Pharmacy.
Professor Cum Principal Department Of Pharmaceutical Chemistry, Annai J.K.K Sampoorani Ammal College Of Pharmacy.
Assistant Professor,Pharmacology,Excel College Of Pharmacy.

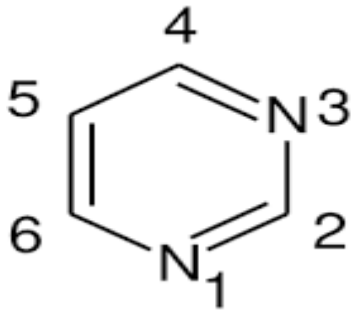
Date of Submission: 10-03-2025 Date of Acceptance: 20-03-2025

ABSTRACT:
A good number of antibacterial drugs are already exists in market but due the changing life style which makes the drugs are infective against those organism. Most of the drugs become inactive against the life threatening microbes because of development of resistant against them. hence in future we require new categories of drugs to overcome this issues. Since most of the pyrimidine compound have antioxidant property. Thus dihydropyrimidinthione one of the derivative of pyrimidine are selected for the study. A number of synthesis of dihydropyrimidinthione has been done under both solvent and solvent free condition, but the antibacterial activity of various derivatives of dihydropyrimidinthione are remain unexplored. Hence in this paper initially the compound are synthesised through solvent free method and the purposed activity are confirmed through docking study in which targeted enzyme are selected and activity are compared with standard like ciprofloxacin followed by that in vitro antibacterial activity are carried out by agar plate method .This paper is focused on antibacterial activity an attempt is made to explore the antibacterial potential of the compound.

Keyword: Ethyl 4-(4-chlorophenyl)-6-methyl-2-thioxo-1234-tetrahydropyrimidine-5-carboxylate), Ciprofloxacin, enzyme, agar plate method, solvent free synthesis.

I. INTRODUCTION:

Pyrimidine is an organic aromatic heterocyclic compound equivalent to pyridine. There are three diazines (six-membered heterocyclic which have two N atoms in the ring) Which has the N atoms at 1 and 3 positions in the ring. The other two diazines are pyrazine which have (N atoms at the position 1 and 4) and pyridazine (N atoms at the position 1 and 2).

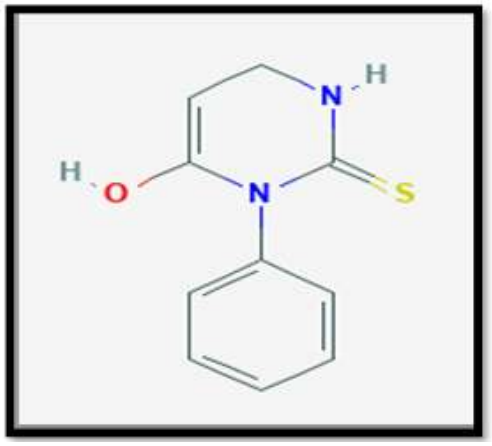


Pyrimidine

Dihyrdopyrimidinthiones, commonly known as Biginelli's compounds, have attained unprecedented attention due to its greater biological, pharmaceutical and therapeutic properties. Dihydropyrimidinthiones have been known as valuable heterocyclic scaffolds due to their versatile bioactivities found in both the synthesized form as well as related marine natural products and have attracted greatest attention recently in synthetic organic chemistry due to their application in the field of drug research and reported various pharmacological and therapeutic properties Such as:

1. Anticancer
2. Antitubercular
3. Antihypertensive
4. Anti inflammatory

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar – Apr 2025, pp: 421-427 www.ijprajournal.com ISSN: 2456-4494

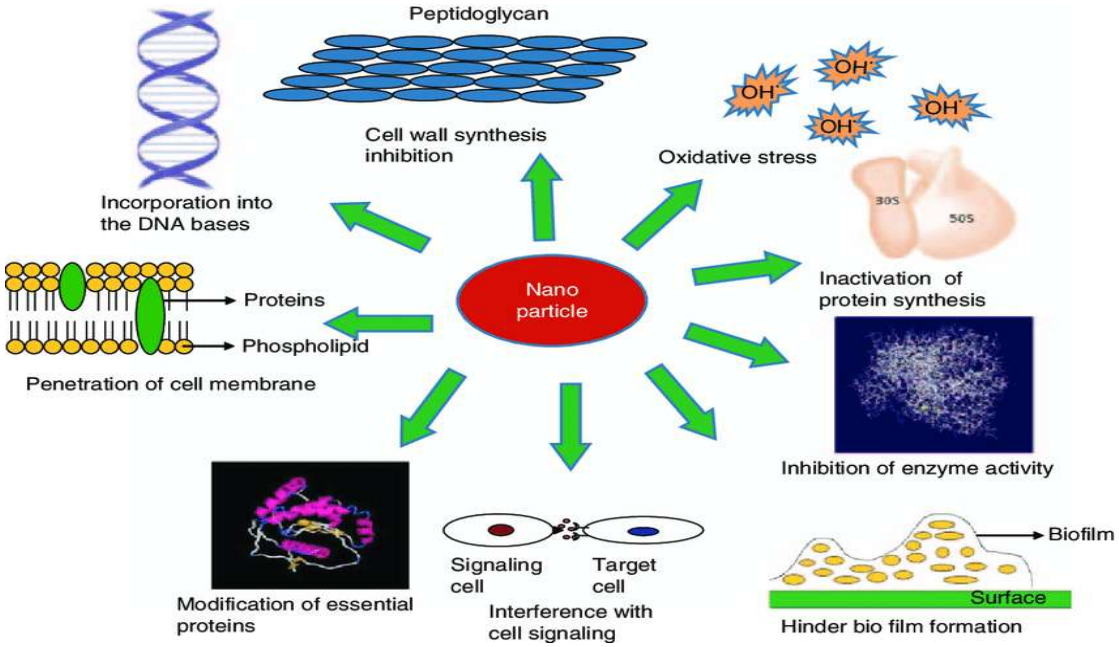


Pyrimidinthione

In organic synthesis, DHPMs have also been extensively employed as starting materials for the synthesis of numerous other heterocyclic derivative.

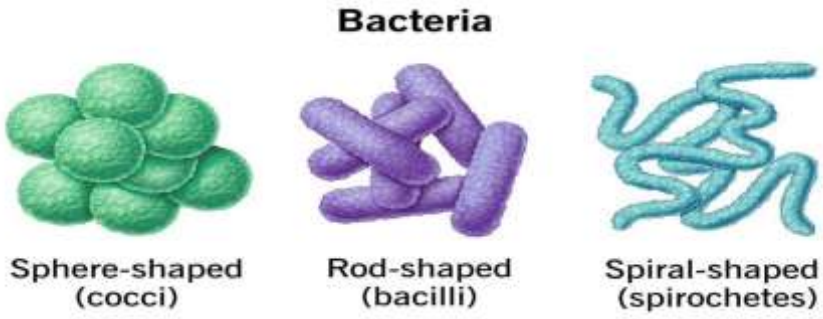
Here Dihydropyrimidinthione whose derivatives having broad spectrum of antibacterial activities have been synthesized by using variation in all the three building blocks. Further novel dihydropyrimidines3 with various important biological properties have been synthesized through effective process under optimal condition employing green catalysts, which should be designed to neglate the use and generation of hazardous substances.

Antibacterial activity is the chemical which either inhibit or kill the growth of bacteria in local level but others nontoxic to surrounding tissue. Antibacterial substance that are targeting the cell wall, membrane, protein synthesis, DNA replication and other part of cell. The spread of bacterial infection is important offensive threat to the living being .The antibiotics are the best standard in the treatment of many bacterial infections. The majority of mechanism is based on the interaction with synthesis of cell wall of bacteria or parasite that preventing the multiplication of the organism.



Where 30% of the cases are bacterial infection which have high treatment value, among them E. coli is the major intestinal microflora causing infection in human. These intestinal pathogenic E. coli produces 08–10% of illness in children and another common opportunistic gram-negative pathogen is Pseudomonas aeruginosa (P. aeruginosa) is the significant cause of nosocomial infection, which leads to a variety of infections such as pneumonia, muscle soreness, bacteremia, and UTI. Patients with weakened immune systems are more prone to P. aeruginosa infection. However, continuous use of antibiotics in day today life to combat the opportunistic infection that are created in an individual may results in Antimicrobial resistant.

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar – Apr 2025, pp: 421-427 www.ijprajournal.com ISSN: 2456-4494



Since Antimicrobial resistance has become a major global health threat during pandemic situation, roughly about 5 million death were associated with AMR in 2019, for example the COVID-19 pandemic enhanced the antibiotic usage and While treatment of COVID-19 with antimicrobials is not effective, because of advanced the development of pathogen resistance. The 2022 Global Burden of Disease survey has determined that E. coli and P. aeruginosa are the major causes (73%) of Antimicrobial resistant related death in 2019. Thus article is focused on pharmacological results of the Dihydropyriminthiones in the area of Anti-bacterial activity.

II. MATERIALS AND METHOD:

PROCEDURE:

A mixture of 0.1 mole of various substituted aldehydes, 0.1 mole of Ethylacetoacetate and 0.1 mole of thiourea without any catalyst under solvent free condition, were placed in a round bottom flask was Shaken for two minutes. The Reaction mixture was then heated under reflux for 2 hours. At the end of the reaction the solid gets deposit. Then, the reaction mixture was allowed to cool and washed with cold water to remove excess of urea. It was filtered and recrystallised using ethanol to afford a pure solid product. The solid was taken out carefully and washed to remove excess of urea with cold water. Then, recrystallized from rectified spirit to afford a pure solid product.

MOLECULAR DOCKING STUDY:

Docking is the process by which two molecules fit together in 3D space. It is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. In this article docking was carried out for sample ethyl 4-(4-chlorophenyl)-6-methyl-2-thioxo-1234-tetrahydropyrimidine-5-carboxylate) and Standard **Ciprofloxacin** by using the chimera software. The Active sites were selected using **PDB sum by Ligplot interactions** where **TYROSYL t-RNA LIGASE SYNTHETASE** has been selected as the targeted enzyme and **PDB ID: 1JIL** will act as the target protein. The molecular interaction between the compound and receptor are viewed by viewed through AUTODOCK 4.20 software.

EVALUATION OF IN VITRO ANTI-BACTERIAL ACTIVITY:

Antibacterial activity of synthesized compounds against gram negative (E.coli and Pseudomonas aeruginosa) bacteria and gram positive bacteria (Bacillus subtilis and MRSA) were investigated in vitro antibacterial using Agar well diffusion method.

INVITRO ANTIBACTERIAL ACTIVITY:

ORGANISM: Escherichia coli (gram negative)
Pseudomonas aeruginosa (gram negative)
Bacillus subtilis **(**gram positive bacteria)
Methicillin - resistant staphylococcus aureus (MRSA) (gram positive bacteria)

CONTROL: Distilled water
CONCENTRATION**:** 10µg/ml
METHOD**:** Well diffusion method.
STANDARD**:** Ciprofloxacin.
SAMPLE**:** Ethyl 4-(4-chlorophenyl)-6-methyl-2-thioxo-1234-tetrahydropyrimidine-5-carboxylate)

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar – Apr 2025, pp: 421-427 www.ijprajournal.com ISSN: 2456-4494

PROCEDURE FOR WELL DIFFUSION TEST:

Inoculum is usually prepared from sub-culture of the organism, instead of from the primary culture. This is because in sensitivity test done on primary culture from bacteria clinical specimens, the number of bacteria in the inoculums cannot be standardized and sometimes the number is so small that results cannot be read properly. Gram staining is to be performed before preparing the inoculums from the sub-culture 4-5 colonies show similar morphology are selected and inoculated into 4-5ml of suitable broth using an inoculums needle/loop. The broth incubated at 35-37^{0}C for 2-5 hours to obtain moderate turbidity diluted with the saline or sterile to obtain turbidity equivalent to that of standard.

METHOD OF INOCULATION:

Inculum is prepared from microbial suspension and placed over the solidified medium with the help of moistened sterile swab. The well is cut on the Petri plates using corkborer with 4mm in diameter. The sample were dissolved in water (10mg/ml) of each sample were diffused on the agar plate using micropipette. The plates were incubated at 37^{0}C for 16-24 hrs.then the microbial growth was determined by measuring the diameter of zone of inhibition.

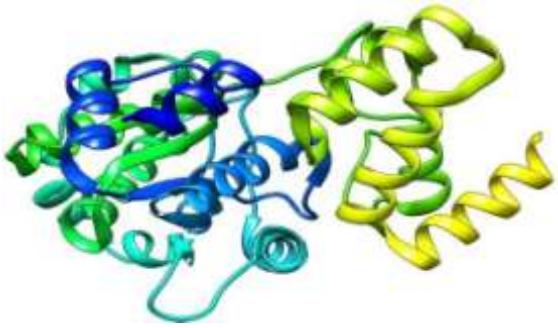
III. RESULT AND DISCUSSION:

In this article, pyrimidine is selected as the core ring for evaluating the antibacterial activity. Initially pyrimidine derivative such as Dihydropyrimidinthione was selected to carry out the docking study as well as invitro antibacterial activity by using ciprofloxacin as standard. The research data has promising effect in antibacterial activity.

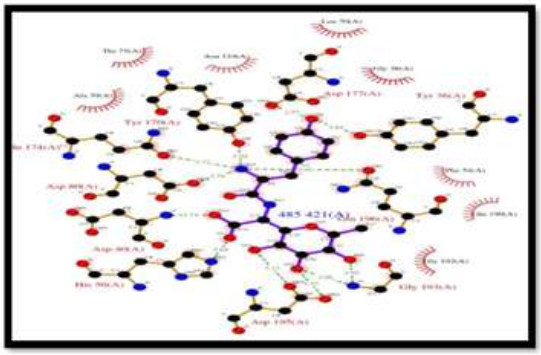
DOCKING STUDY:

In this article docking was carried out for sample ethyl 4-(4-chlorophenyl)-6-methyl-2-thioxo-1234-tetrahydropyrimidine-5-carboxylate) and Standard (Ciprofloxacin) by using the chimera software. The Active sites were selected using PDB sum by Ligplot interactions where TYROSYL t-RNA LIGASE SYNTHETASE has been selected as the targeted enzyme and PDB ID: 1JIL will act as the target protein. The molecular interaction between the compound and receptor are viewed by viewed through AUTODOCK 4.20 software.

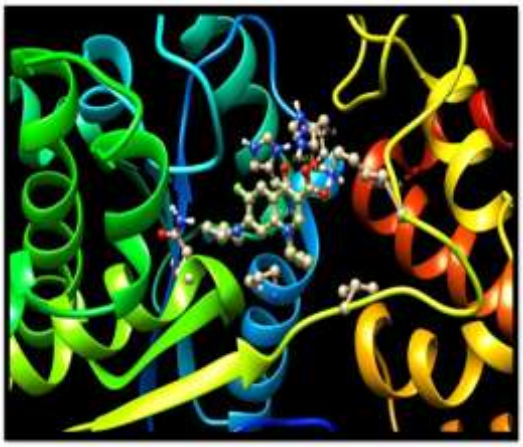
MOLECULAR DOCKING FOR ANTI-MICROBIAL ACTIVITY
TARGET ENZYME: TYROSYL t-RNA LIGASE SYNTHETASE
TARGET PROTEIN: PDB ID: 1JIL



ACTIVE SITE: Active sites were selected using PDB sum by Ligplot interaction**s.**

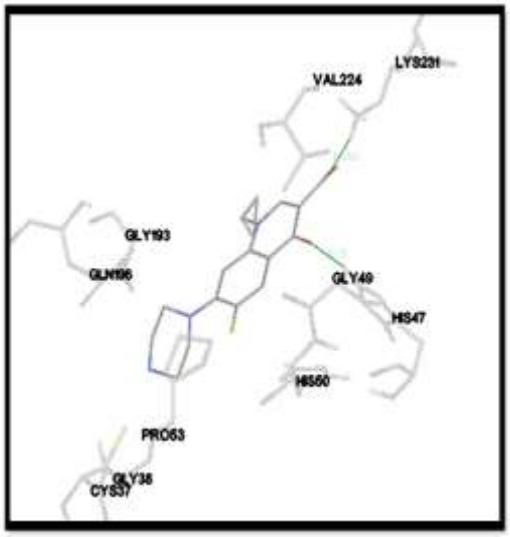


GRID BOX SELECTION-60*60*60

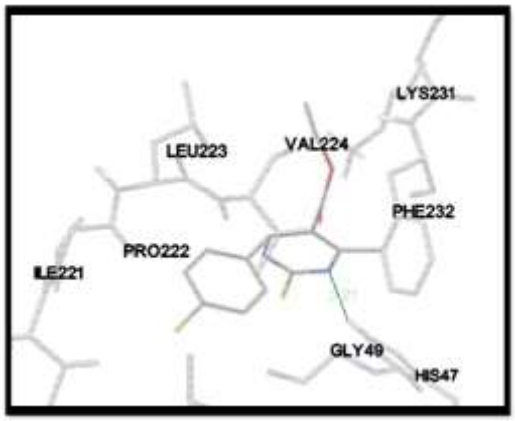


Molecular interaction for Control (Ciprofloxacin) in the active site of 1JIL

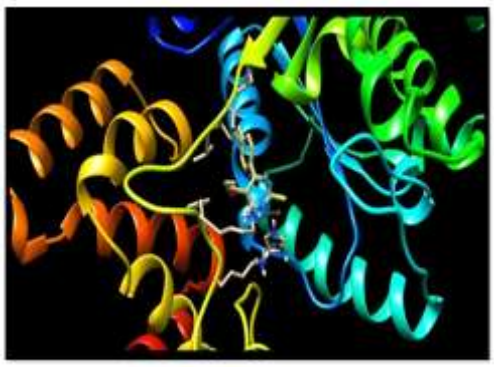
International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar – Apr 2025, pp: 421-427 www.ijprajournal.com ISSN: 2456-4494



Molecular interaction for Ciprofloxacin in the active site of 1JIL viewed through AUTODOCK 4.20



Molecular interaction for Compound A in the active site of 1JIL viewed through AUTODOCK 4.20 software



Molecular interaction for Compound A in the active site of 1JIL

Compound	H-Bond Interaction	H-Bond Distance (Å)	Non-Bonding Interactions	Binding Energies (Kcal/Mole)
ethyl 4-(4-chlorophenyl)-6-methyl-2-thioxo-1234-tetrahydropyrimidine-5-carboxylate	UNK N : VAL 224 :A	2.21	Ser 194, Leu 223, Phe 232, Asp 195, Gly 193, Leu 52, Pro 32	-6.83
CIPROFLOXACIN	UNK O: LYS 231:A UNK O: HIS 47:A	1.841 1.641	Phen 92, Ser 330, Arg 227, Pro 336, Pro 326, Ile 338, Ser 382, Asp 384, Phe 383	**-7.32**

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar – Apr 2025, pp: 421-427 www.ijprajournal.com ISSN: 2456-4494

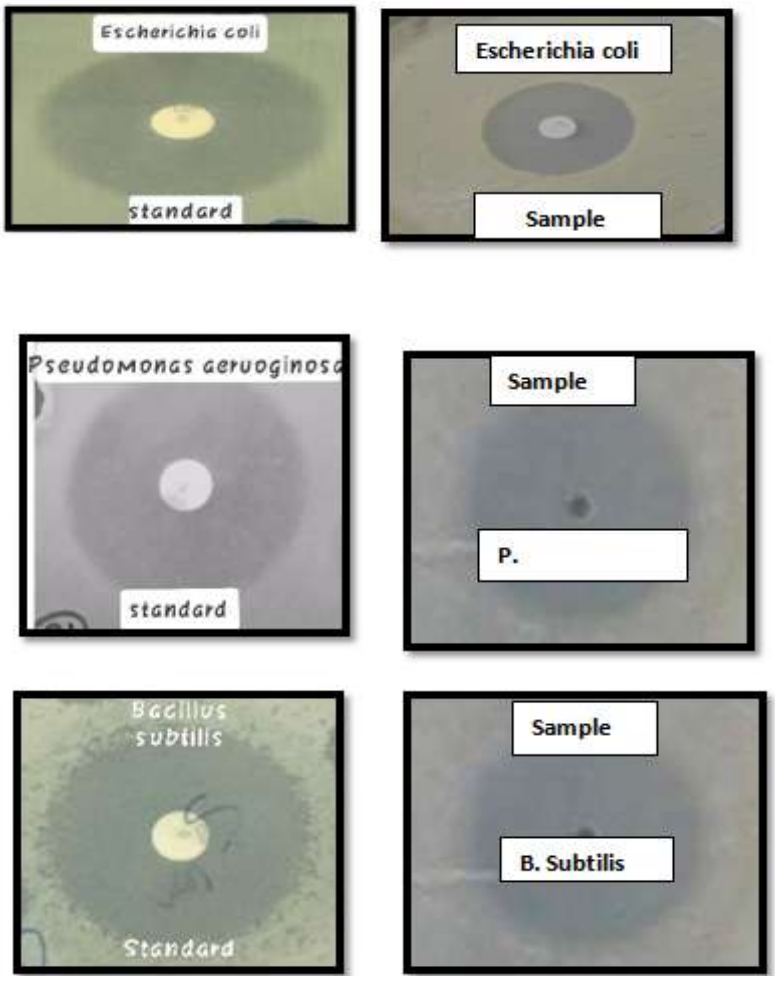
MOLECULAR INTERACTIONS OF LIGAND COMPOUNDS WITH PROTEIN IN 1JIL

Compound Code	Binding Energy (Kcal/Mol)	Ligand Efficiency	Inhibitory Constant (µM)	Vdw - Hb Desolvation Energy (K Cal/Mol)
NAME	-6.83	-0.34	9.92	-8.02
CIPROFLOXACIN	**-7.32**	-0.22	36.49	-8.04

ENERGY MINIMIZATION TABLE

EVALUATION OF IN VITRO ANTI-BACTERIAL ACTIVITY:

Antibacterial activity of synthesized compounds against gram negative (E.coli and Pseudomonas aeruginosa) bacteria and gram positive bacteria (Bacillus subtilis and MRSA) were investigated in vitro using well diffusion method were ciprofloxacin as standard. The synthesized compounds were taken for their in vitro antibacterial activity against gram positive (Bacillus subtilis and MRSA) and gram negative (E.coli and Pseudomonas aeruginosa) organisms using well diffusion method at the concentration of 100µg/ml.



International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar – Apr 2025, pp: 421-427 www.ijprajournal.com ISSN: 2456-4494

ANTIBACTERIAL ACTIVITY OF COMPOUNDS AGAINST ESCHERICHIA COLI

COMPOUND CODE / MICROBES	ZONE OF INHIBITION			
	Escherichia coli	Pseudomonas aeruginosa	Bacillus subtilis	**MRSA**
ethyl 4-(4-chlorophenyl)-6-methyl-2-thioxo-1234-tetrahydropyrimidine-5-carboxylate.	12mm	**15 mm**	11mm	11mm
CIPROFLOXACIN	**22mm**	**21mm**	**20mm**	-

IV. CONCLUSION

In the present study the novel Dihydropyrimidinthione derivative ethyl 4-(4-chlorophenyl)-6-methyl-2-thioxo-1234-tetrahydropyrimidine-5-carboxylate) has showed good binding energies against target enzymes for antibacterial study. Based on the docking score invitro antibacterial activity ethyl 4-(4-chlorophenyl)-6-methyl-2-thioxo-1234-tetrahydropyrimidine-5-carboxylate) are evaluated using agar well diffusion.method at the concentration of100g/ml. where ciprofloxacin is used as standard in the activity and Escherchia.coli,pseudomonas arginosa,MRSA are selected as a bacterial strain against which antibacterial activity performed.

From the result it is clearly showed that Dihydropyrimidinthione derivative ethyl 4-(4-chlorophenyl)-6-methyl-2-thioxo-1234-tetrahydropyrimidine-5-carboxylate) has promising antibacterial activity against the selected strain. In future these ethyl 4-(4-chlorophenyl)-6-methyl-2-thioxo-1234-tetrahydropyrimidine-5-carboxylate) could be used to treat the bacteria showing resistance against existing standard drugs.

REFERENCE:

[1]. Brown, D. J.; Evans, R.F.; Cowden, W. B.; Fenn, M. D. (1994). The Pyrimidines. New York: John Wiley & Sons. pp. 5–6.

[2]. Nevagi R. J and Narkhede H. I: Novel dihydropyrimidine derivatives as antibacterial agents.

[3]. Scholars Research Library2014; 6(3):135-139.

[4]. Hojati S. F,Gholizadeh M, Haghdoust M, and Shafiezadeh F: 1,3-dichloro-5,5dimethylhydantoin as a novel and efficient homogeneous catalyst in Biginelli reaction.Bulletin of the korean chemical society 2010; 31, 3238-3239.

[5]. **chohan, Z. H., & Perveen, S. (2005).** "Synthesis and antimicrobial activity of dihydropyrimidine derivatives." Bioorganic & Medicinal Chemistry Letters, 15(15), 3343–3346.

[6]. **Hasaninejad, A., & Naghdi, T. (2013).** "Synthesis, characterization and antibacterial activities of novel dihydropyrimidine derivatives." European Journal of Medicinal Chemistry, 67, 389–395.

[7]. **Silver, L. L. (2011).** "Antibacterial drug discovery: A review." Future Microbiology, 6(5), 605–624