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In Silico Design and Solvent Free Synthesis of Some Novel Dihydropyrimidinthione Derivatives and Study of its Antimicrobial Activity

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Abstract

According to WHO, 700,000 people were affected by antibiotic resistance per year and it becomes a serious threat to global health. Keeping in view this observation and emerging need of new drug candidates to overcome the antibiotic resistance and also to fight against the emerging diseases, the present research study focussed on to develop some novel antimicrobial agents. The study involved *Insilico* design and solvent free synthesis of some novel dihydropyrimidinthione derivatives and study of its antimicrobial activity. The purity of the synthesized compounds was confirmed by TLC and melting point determination. The structures of the synthesized compounds were characterized, predicted using CHEMSKETCH, CHEM DRAW and MARVIN SKETCH software. Drug likeness properties were studied using MOLINSPIRATION software. All the synthesized compounds obey the Lipinski rule of *Five*. In*silico* ADME studies were performed using SWISS ADME online web tool, toxicity profile studied using OSIRIS property explorer software for all the designed compounds only compound F was found to be tumorogenic . The docking study was performed for all thedesigned compounds against the targeted enzyme Tyrosyl t-RNA Ligase Synthetase (1JIL) using AUTODOCK 4.2 software. The docking results showed Compound A, C and D produced good binding affinity and Compound B showed significant binding score (-8.30 kcal/mol) compared to standard ciprofloxacin (-7.32 kcal/mol) Based on the docking score compounds A, B, C, and F were screened for their *in vitro* antibacterial activity against gram positive and gram-negative organisms using well diffusion method at the concentration of 100µg/ml. Compound A, B, C and D showed the good antibacterial activity compared to standard ciprofloxacin at the concentration of 100µg/ml.

Keywords: Solvent free synthesis, SWISS ADME, MOLINSPIRATION, OSIRIS, AUTODOCK 4.2.

Introduction

Antibiotic resistance is all the time more recognized as a serious and permanent public health concern and is usually considered to be a consequence of wide use and misuse of antibiotics(1). Antibiotic resistance drug discovery and development is one of the most essential and rapidly changing avenues for medicinal chemist. Despite a large number of antibiotics and for medicinal use, the treatment of infectious diseases remains an important and challenging problem(2). This is because of a combination of factors including emergence of resistance to current antimicrobial therapy and rapid increase of primary and opportunistic fungal infections in immune compromised patients like those suffering from immunodeficiency syndrome (aids) or undergoing anticancer therapy and organ transplantation. So, the drug resistivity of microbes has been increased enormously. To combat this

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antimicrobial resistance, it is necessary to develop a new and effective antimicrobial drug. Dihydropyrimidinthione is one of the promising candidates attracted substantial attention of the medicinal chemists(3). Dihydropyrimidinthione were reported as therapeutic leads to develop newer and effective pharmacophores with enhanced and a versatile range of medicinal activities like such as antiviral(5), anticancer(3), antibacterial(3), antituberculosis(4), antihypertensive(6), antiarrythmic activities(7). In keeping in view, the biological significance and medicinal utility of dihydropyrimidinthione derivative, the present study involved solvent free synthesis, molecular docking and study of some novel dihydropyrimidinthione derivatives against Tyrosyl t-RNA Ligase Synthetase as a target enzyme.Green chemistry approach under solvent free condition Simplify and improve conditions have been used traditionally to carry out the conventional Biginelli reaction involves three-component one-pot condensation of an aldehyde, β-ketoester and urea or thiourea in ethanol under strong acidic condensation HCl for the synthesis of dihydropyrimidinthione derivatives by green chemistry approach. These reactions were performed by three-component condensation of different types of an aldehyde (benzaldehyde, acetaldehyde, furfural, cinnamaldehyde, and salicaldehyde etc.,), ethyl acetoacetate, and urea or thiourea at reflux temperature under solvent-free conditions with catalyst (scheme II) or without catalyst (scheme I) to afford the corresponding dihydropyrimidinthione in good yield (76–96%).

Material and Methods

All the chemicals and reagents used in experimental reactions were Analytical Grade. The melting point of the titled compounds were determined using one end sealed open capillary tube method and are uncorrected. The purity of the synthesized compounds was also checked by TLC on a pre coated silica gel using chloroform: methanol (9:1) and spots were visualized using UV- chamber. The ^{1}H NMR and C^{13} NMR spectra were predicted using CHEM DRAW software. The Mass spectra were also predicted by MARVIN SKETCH software. The compounds were designed by Molecular docking study using AUTODOCK 4.2 software. All the synthesized compounds were evaluated for *in silico* ADME studies using SWISS ADME online and for its toxicity profile using OSIRIS property explorer software.

Synthesis:

***General Procedure for the Synthesis of Titled Compounds*:** A mixture of 0.1 mole of substituted aldehydes, 0.1 mole of Ethylacetoacetate and 0.1 mole of thiourea with catalyst or without using any catalyst under solvent free condition, were taken in a round bottom flask, the reaction mixture was continuously shaken for two minutes, then heated under reflux for 2 hours, as the progress of the reaction was observed a solid compound was started to deposit. The completetion of reaction was monitored by TLC using solvent system [chloroform: methanol (9: 1). 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.

Scheme of Synthesis:

***Scheme – I*:** 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 taken in a round bottom flask was shaken continuously for two minutes. The Reaction mixture was then heated under reflux for 2 hours, a solid product was started to deposit. The completetion of reaction was monitored by TLC using solvent system [chloroform: methanol (9: 1). 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 with cold water to remove excess of urea. Then, recrystallized

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from rectified spirit to afford a pure solid product (Figure.1.0).

***Scheme – II*:** To a mixture of various substituted aromatic aldehydes (0.1 mole), ethyl acetoacetate (0.1 mole), thiourea (0.1 mole) and **[Btto][p-TSA] (**0.15 mmoL) was heated at 90^0C for 30 minutes under solvent free condition with magnetic stirring. The completion of reaction was monitored by TLC. After cooling, the reaction mixture was poured on to crushed ice and stirred for 5 min. The separated solid was filtered and washed with cold water thoroughly. Then, recrystallized the synthesized products from ethanol to afford the pure product (Figure.1.1).

Physical and Spectroscopic Data of Synthesized Products

***Physicochemical Properties*:** The Physicochemical properties of the synthesized compounds were also determined. The purity of the synthesized compounds was determined by TLC and melting point determination. All the synthesized compounds obey's the Lipinski's rule of five. So, it has a good oral bioavailability. Determination of the Elementary analysis for all the synthesized compounds using CHEMKETCH software.

Spectroscopic Data for the Synthesized Products: The structures were characterized using predicted spectrum from CHEMSKETCH, CHEM DRAW and MARVIN SKETCH software. Drug likeness properties were studied using MOLINSPIRATION software.

A [ethyl 4- (4-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate]

^{13}C-NMR; δ (ppm) = 166 (-C), 61.7 (-CH_2), 174.5 (-C), 141.3 (-C), 128.3 (-CH), 15.6 (-CH_3), 54.6 (-CH_2), ^{1}H NMR; δ ppm: 4.19 (s,1H,-CH), 2.0 (s, 1H, NH), 4.59 (s, 3H, CH_3), 7.00 (s,1H,CH), 7.15 (s,1H,CH), 1.30 (s,2H,CH_2), Mass (m/z): 310

B [ethyl 4- (3-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate]

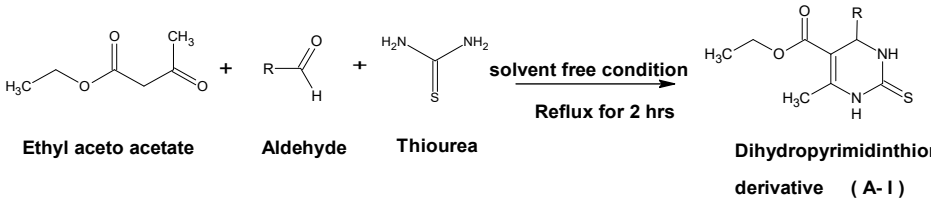

Fig 1.0. Solvent Free Synthesis of Dihydropyrimidinthione Derivative without Catalyst

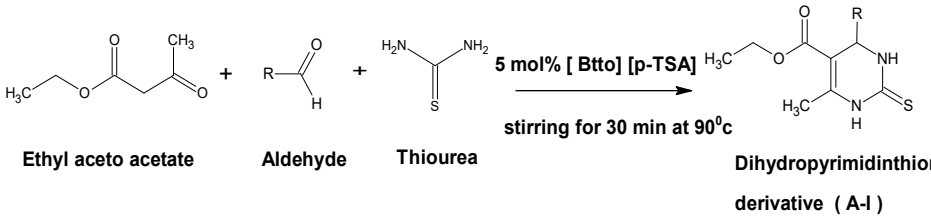

Fig 1.1 Solvent Free Synthesis of Dihydropyrimidinthione Derivative using Catalyst (p-TSA)

Novel Dihydropyrimidinthione Derivatives

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^{13}C-NMR; δ (ppm) = 167.2 (C=O), 61.7 (-CH_2), 104.2 (C=C), 130 (-C), 54.6 (-CH_2), 15.6 (-CH_3), 160.3 (-C=C) ^{1}H NMR; δ ppm: 2.0 (s,1H,NH), 1.30 (s,3H,CH_3), 4.19 (s,1H,-CH), 1.71 (s,3H,CH_3), 7.53 (tdd,1H-CH), 4.59 (s, 3H, CH_3), Mass (m/z): 310.

C [ethyl 4- (2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate]

^{13}C-NMR; δ (ppm) = 167.2 (C=O), 45.5 (-CH_2), 104.2 (-C), 142.8 (-C), 160.3 (-C), 180.4 (C=S), 142.8 (-C), 61.70 (-CH_2), ^{1}H NMR; δ ppm: 4.19 (s,2H,-CH_2), 2.0 (s,1H, -NH), 4.59 (tdd,1H,-CH), 1.30 (s,3H,-CH_3), 7.0 (s,1H,-CH), 1.71 (s,3H,CH_3), Mass (m/z): 310.

D [ethyl 6-methyl-4- (2-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate]

^{13}C-NMR; δ (ppm) = 167.2 (C-O), 46.0 (-CH2), 104.2 (C=C), 180.4 (C=S), 61.70 (-CH_2), 142.8 (-C), 137.5 (C=C), 1H NMR; δ ppm: 4.19 (s,2H,-CH_2), 2.0 (s,1H,-NH), 4.59 (dd,2H,CH-NH), 8.07 (s,1H,-C), 1.30 (tdd,3H,-CH_3), 7.53 (tdd,1H-CH), 7.32 (s,1H,-CH), 1.71 (s,3h,CH_3), Mass (m/z): 321.

E [ethyl 4- (4-hydroxy-3-methoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate]

^{13}C-NMR; δ (ppm) = 167.2 (C-O), 54.9 (-CH2), 104.2 (C=C), 180.4 (C=S), 61.70 (-CH_2), 151.2 (-C), 160.3 (-C),1H NMR; δ ppm: 4.19 (s,2H,-CH_2), 2.0 (s,1H,-NH), 4.59 (dd,2H,CH-NH), 6.40 (s,1H,-C), 1.30 (tdd,3H,-CH_3), 6.45 (tdd,1H-CH), 5.0 (s,1H,-OH), 1.71 (s,3h,CH_3), Mass (m/z): 322.

F [ethyl 4-[4- (dimethylamino)phenyl]-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate]

^{13}C-NMR; δ (ppm) = 167.2 (C-O), 54.6 (-CH2), 104.2 (C=C), 180.4 (C=S), 61.70 (-CH_2), 151.2 (-C), 160.3 (-C), ^{1}H NMR; δ ppm: 4.19 (s, 2H, -CH_2), 2.0 (s,1H,-NH), 4.59 (dd,2H, CH-NH), 6.47 (s,1H,-CH), 1.30 (tdd,3H,-CH_3), 6.88 (s,1H.-CH), 6.45 (tdd,1H-CH), 5.0 (s,1H,-OH), 1.71 (s,3h,CH_3), 2.85 (s,3H,-CH_3), Mass (m/z): 319.

G [ethyl 4- (furan-2-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate]

^{13}C-NMR; δ (ppm) = 167.2 (C-O), 55.8 (-CH2), 104.2 (C=C), 180.4 (C=S), 61.70 (-CH_2), 152.5 (-C), 160.3 (-C), 106.7 (-CH) ^{1}H NMR; δ ppm: 4.19 (s,2H,-CH_2), 2.0 (s, 1H, -NH), 4.82 (dd,2H,CH-NH), 6.06 (s,1H,-CH), 1.30 (tdd,3H,-CH_3), 6.24 (s,1H.-CH), 7.28 (tdd,1H-CH),1.71 (s,3h,CH_3), 2.85 (s,3H, -CH_3), Mass (m/z): 266.

H [ethyl 4- (2-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate]

^{13}C-NMR; δ (ppm) = 167.2 (C-O), 44.4 (-CH2), 104.2 (C=C), 180.4 (C=S), 61.70 (-CH_2), 122.9 (-C), 160.3 (-C), 128.4 (-C) ^{1}H NMR; δ ppm: 4.19 (s,2H,-CH_2), 2.0 (s,1H,-NH), 4.59 (dd,2H,CH-NH), 6.70 (s,1H,-CH), 1.30 (tdd,3H,-CH_3), 6.89 (s,1H.-CH), 6.90 (tdd,1H-CH),1.71 (s,3h,CH_3), 5.00 (s,3H,-OH), Mass (m/z): 266.

I [ethyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate]

^{13}C-NMR; δ (ppm) = 167.2 (C-O), 54.6 (-CH2), 104.2 (C=C), 180.4 (C=S), 61.70 (-CH_2), 122.9 (-C), 160.3 (-C), 128.6 (-C) ^{1}H NMR; δ ppm: 4.19 (s,2H,-CH_2), 2.0 (s,1H,-NH), 4.59 (dd,2H,CH-NH), 7.14 (s,1H,-CH), 1.30 (tdd,3H,-CH_3), 7.06 (s,1H.-CH), 6.90 (tdd,1H-CH),1.71 (s,3h,CH_3), Mass (m/z): 276.

In Silico Studies

Evaluation of In Silico Adme Properties for the Synthesized Compounds Using Swiss Adme Software:

***Swiss ADME Tool*:** The pharmacokinetics and drug likeness prediction of compounds were performed online on Swiss ADME tool. The online prediction was done to check the compound were inhibitors of cytochrome P450.In addition to the pharmacokinetic properties such as Gastrointestinal absorption, Blood-Brain Barrier penetration, Skin Permeation, synthetic associability and drug-likeness prediction like

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Lipinski, Ghose and Veber rules and bioavailability score were also assessed.

***Bioavailability Radar*:** The drug-likeness of a molecule can be rapidly assessed from the Bioavailability Radar. The pink colored zone is the suitable physiochemical space for oral bioavailability and the radar plot of the molecule has to fall entirely in the zone to be considered drug-like. The pink area represents the optimal range of each property LIPO (Lipophilicity): -0.7 < XLOGP 3 < +5.0; SIZE: 150 g/mol< MW < 500 g/mol; POLAR (polarity): 20Å^2< TPSA < 130Å^2; INSOLU (Insolubility): 0 < Log S (ESOL) < 6; INSATU (Insaturation): 0.25 < Fraction of Csp3 < 1; FLEX (Flexibility): 0 < Num. rotatable bonds < 9. From the Swiss ADME prediction output, it is evident that all the compounds have the optimal range of all the six properties, enabling them to be considered to possess significant chemotherapeutic potentials.

***Pharmacokinetics Properties*:** All the designed compounds (Fig.1.2 – 2.1) were observed with high intestinal absorption and hence should permeate quite easily across the intestinal lining and available for the cell membrane. drugs that act in the CNS need to pass over the blood brain barrier (BBB) to reach their molecular target. however, little or no BBB permeation might be required for drug

Compound - A

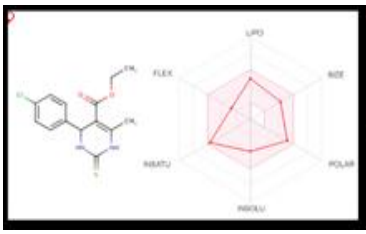

Fig 1.2

Compound - B

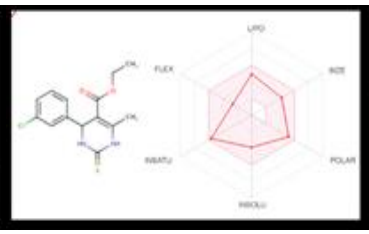

Fig 1.3

Compound - C

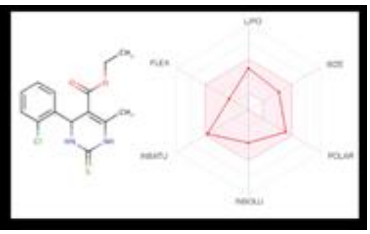

Fig 1.4

Compound - D

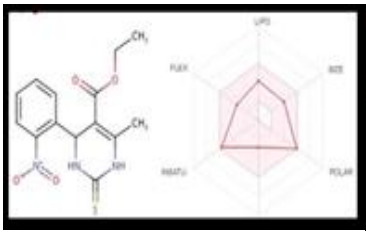

Fig 1.5

Compound - E

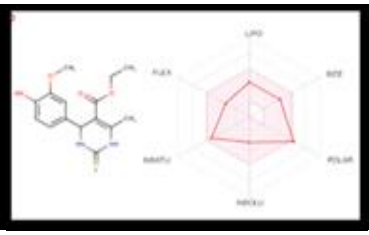

Fig 1.6

Compound - F

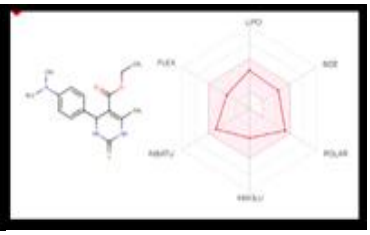

Fig 1.7

Compound - G

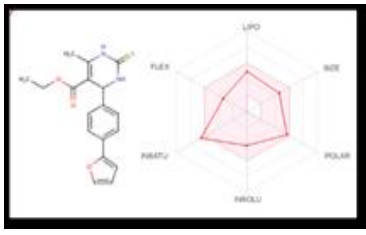

Fig 1.8

Compound - H

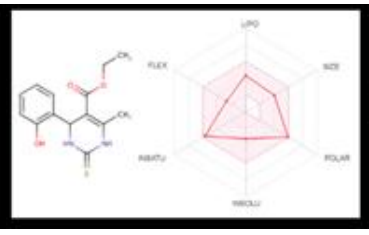

Fig 1.9

Compound - I

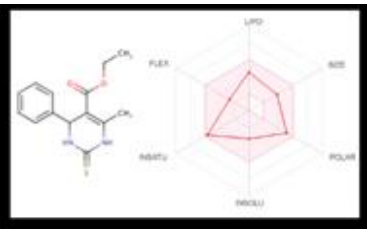

Fig 2.1

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molecules with a peripheral target, so as to avoid CNS side effects.

The Blood-brain barrier (BBB) permeation expresses the relative affinity of the drug for the blood or brain tissue. It was observed that all the compounds predicted have no blood-brain barrier penetration and hence free from CNS side effects.

***BOILED-Egg Model*:** The Brain or Intestinal permeation method (BOILED-Egg) is an intuitive graphical method to accurately predict the passive human gastrointestinal absorption (HIA) and brain permeability (BBB). This classification model relies on the descriptors: WLOGP and TPSA values, for computing lipophilicity and corresponding polarity of small molecules. The egg-shaped classification plot includes the yolk (i.e. the physicochemical space for highly probable brain permeation) and the white (i.e. the physicochemical space for highly probable passive absorption by the gastrointestinal tract). The outside grey region stands for molecules with properties implying predicted low absorption and limited brain penetration. from the boiled-egg plots, it has been observed that all the compounds were spotted in the white yolk attributed to highly probable HIA absorption Fig. 2.2.

Molecular Docking:

***Ligand Preparation*:** Structures of molecules were drawn using ACD Labs Chemsketch 2017 V.2.1 and saved in mol format. Open Babel V.2.4.1 mol format is used for converting formats (mol to pdb). UCSF Chimera V.1.13.1rc was used for optimization and minimizing structures by setting default options, i.e., steepest descent 100 steps, and conjugate gradient 10 steps. On adding hydrogens and assigning Gasteiger's charges, the net charge on the molecules is displayed. On saving the work, molecules were processed for docking using AutoDock Tool V.1.5.6.

***Protein Preparation*:** Proteins were selected from PDB (w3.rcsb.org) and initially processed to remove solvent molecules, heteroatoms, and other non-standard residues. AutoDock Tool V.1.5.6 was used to prepare protein for docking by adding polar hydrogen, merging non-polar hydrogenand assigning Kollman's charges.

***Grid Generation*:** AutoDock Tool is an interactive graphical tool for coordinate preparation, docking, and analysis.Preparation of coordinate files is the most important step of the process, as it affects docking quality. The three-dimensional (3D) grid box was created by AutoGrid algorithm to evaluate the binding energies on the macromolecule coordinates. Using AutoGrid, the grid maps expressing the intact ligand in the docking target site were

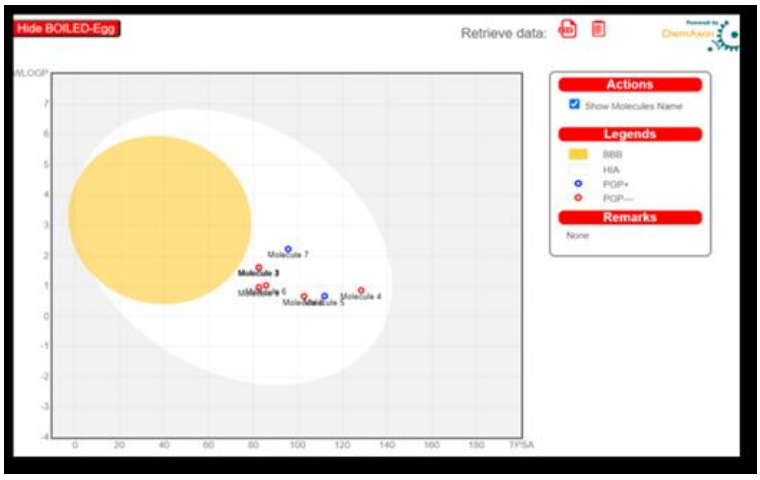

Fig 2.2

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calculated. The 3D grid box with 100 grid size (*x, y, z*) was created with a spacing of 0.375. The selected protein and ligand in PDBQT format were chosen, followed by the generation of GPF (grid parameter file), after which Running AutoGrid provided GLG (grid log file).

***Docking*:** AutoDock V.4.2.6 is a computational docking program based on an empirical free energy force field andrapid Lamarckian genetic algorithm search method. In AutoDock, the overall docking energy of a given ligand molecule is expressed as the sum of intermolecular interaction energies including van der Waals attractive and repulsive energies, H-bond interaction energy, coulombic electrostatic energy, and the internal steric energy of the ligand. The selected protein and ligand on Autogrid options were subjected to docking, followed by generation of DPF (docking parameter file). Running AutoDock provides DLG (docklog file). By default, ten best conformations of protein-ligand interactions were resulted by AutoDock along with binding energy values, inhibition constant (predicted) and H-bond interactions. The docking results are presented below in Tables 1.4 and 1.5. The complexes with good binding energy values were built and subjected to LigPlot+ V.2.1 for visualizing interactions Fig.2.4. The typical interactive patterns are shown in Figs.2.6, 2.7, 2.8.

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.**

Standard: Ciprofloxacin

***Toxicity Profile by Osiris Property Explorer*:**

All the synthesized compounds were studied for its toxicity profile using Osiris Property Explorer software**.** The results

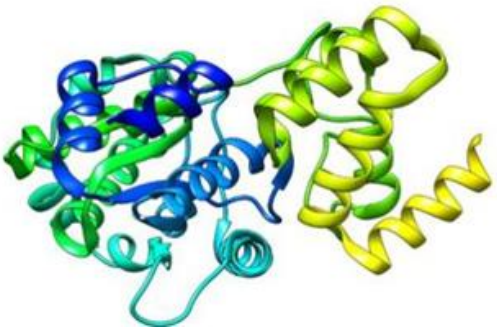

Fig 2.3

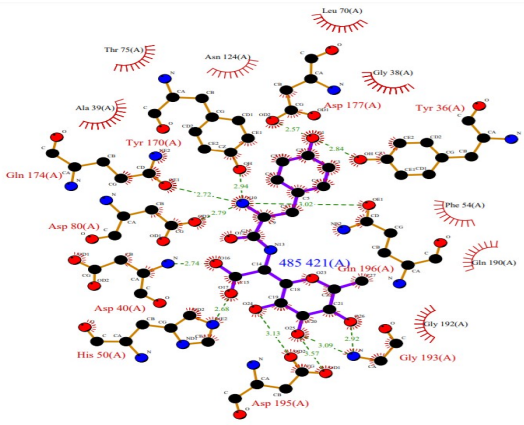

Fig 2.4

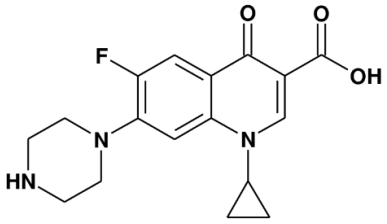

Fig 2.5

Docked Poses Of Standard, Compound B And C in the Binding Pocket with Tyrosyl T-RNA Ligase Synthetase (PDB ID: 1JIL) and Yellow Dashed Lines Represents Hydrogen Bond Interactions

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showed that the most of the studied compounds were found to be non-toxic or low

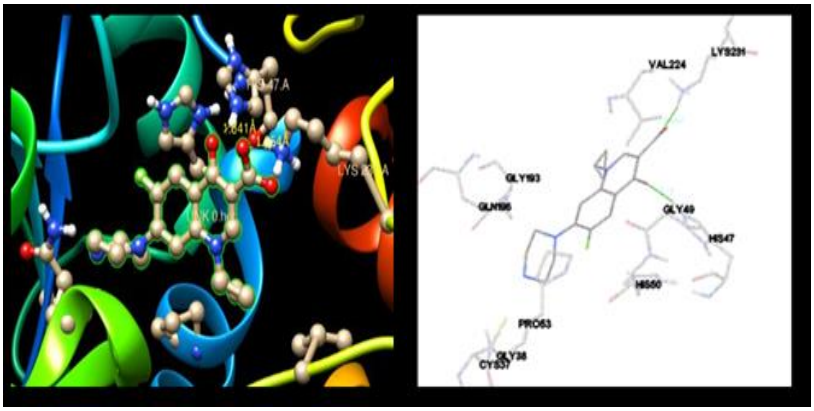

Fig 2.6 Standard (Ciprofloxacin)

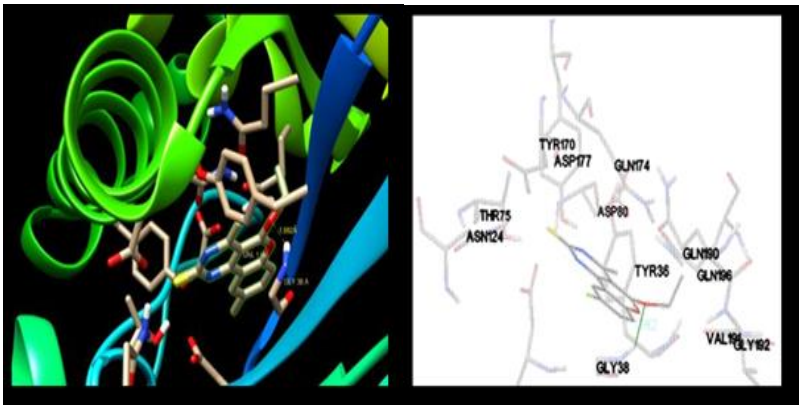

Fig 2.7 Compound B

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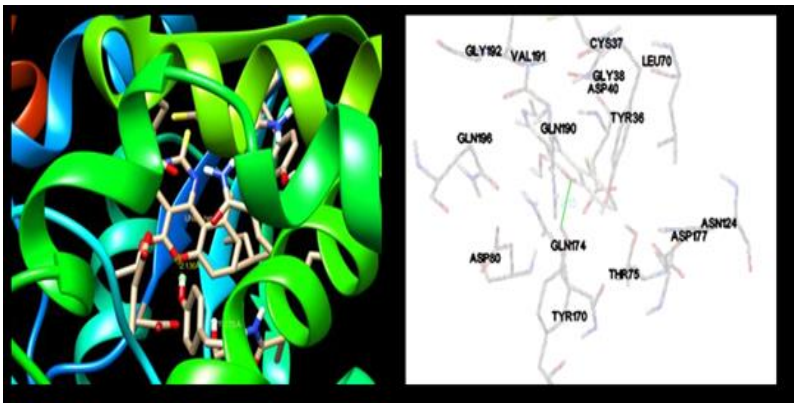

Fig 2.8 Compound C

risk except compound F which showed Tumorogenic effect.

In Vitro Antimicrobial Study

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.

The evaluation can be done by the following methods.

- Turbidimetric method.
- Agar streak method
- Serial dilution method.
- Agar diffusion method.

In agar diffusion methods there are about two types of techniques;

- Agar well diffusion
- Agar disc diffusion

***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: 10 µg/ml

Method: Well diffusion method.

- All the synthesized compounds were screened 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. Compound C showed significant antibacterial activity against *E. coli* (17mm), *Pseudomonas aeruginosa* (19mm), *Bacillus subtilis* (15mm) and *MRSA (*14mm) respectively.
- Among the tested compounds, compound C produces a very good antibacterial activity.
- Compound A, B, C and F showed the good antibacterial activity against MRSA compared to standard ciprofloxacin.

Results

1. Chemistry and Synthesis

➢ A series of nine novel dihydropyrimidinthione derivatives were synthesized by condensing ethyl aceto acetate with various aromatic aldehydes under solvent free condition using catalyst (p-TSA).

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- The reaction has also been carried out without using catalyst.
- The progress of reaction were checked by thin layer chromatography using the solvent system chloroform: methanol (9:1).
- The scheme details has been shown in Table.1.0

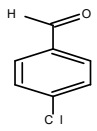
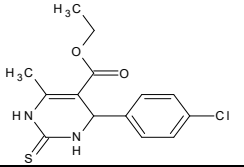
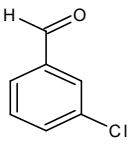
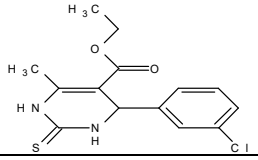
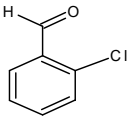
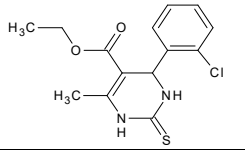
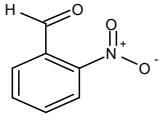
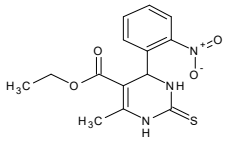
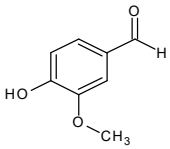
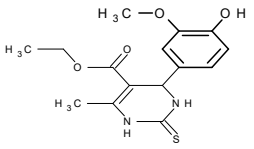
***Scheme Details*:** The purity of the synthesized compounds were characterized by melting point determination and are uncorrected. The physiochemical properties of the synthesized compounds were studied and has been depicted in the Table 1.1 given below:

The chemical structures of synthesized compounds were characterized using PREDICTED NMR and MASS SPECTRUM by the software CHEM DRAW, CHEM SKETCH and MARVIN SKETCH softwares for elemental analysis, NMR and Mass spectra respectively.

***2. In Silico Studies*:** The preliminary QSAR study or drug likeness of the synthesized compounds were studied using MOLINSPIRATION software was shown in the Table 1.2 and 1.3 and the parameters obeys the Lipinski rule of five. Hence, the synthesized compounds have a good oral bioavailability.

***QSAR, Drug Likeness and Docking Results*:** Molecular docking studies of Tyrosyl

Table 1.0

Compound Code	R	Structure
A [ethyl 4- (4-chlorophenyl)- 6-methyl-2-thioxo-1,2,3,4- tetrahydropyrimidine -5-carboxylate]		
B [ethyl 4- (3-chlorophenyl) -6-methyl-2-thioxo- 1,2,3,4-tetrahydropyrimidine -5-carboxylate]		
C [ethyl 4- (2-chlorophenyl) -6-methyl-2- thioxo-1,2,3,4- tetrahydropyrimidine-5-carboxylate]		
D [ethyl 6-methyl- 4- (2-nitrophenyl)- 2-thioxo-1,2,3,4-tetrahydropyrimidine -5-carboxylate]		
E [ethyl 4- (4-hydroxy- 3-methoxyphenyl)-6-methyl- 2-thioxo-1,2,3,4- tetrahydropyrimidine-5-carboxylate]		

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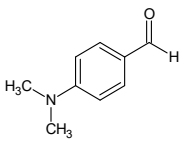
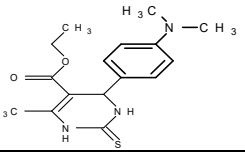
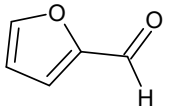
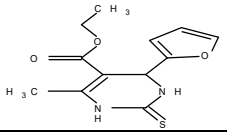
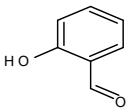
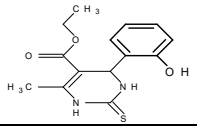
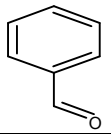
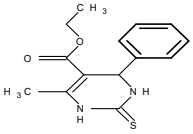
F [ethyl 4-[4- (dimethylamino) phenyl]-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate]		
G [ethyl 4- (furan-2-yl)-6-methyl-2 -thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate]		
H [ethyl 4- (2-hydroxyphenyl) -6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate]		
I [ethyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate]		

Table 1.1. Physicochemical Properties

Compound Code	Molecular Formula	Molecular Weight (gm/mol)	Percentage Yield (%)	Melting Point (^{0}C)	Log P	RF Value
A	$C_{14}H_{12}O_2N_2SCl$	310.81	83.3	206	3.54	0.64
B	$C_{14}H_{12}O_2N_2SCl$	310.81	96.7	180	3.75	0.88
C	$C_{14}H_{12}O_2N_2SCl$	310.81	97.6	220	3.59	0.64
D	$C_{14}H_{12}O_4N_3S$	321.36	96.71	170	2.82	0.52
E	$C_{15}H_{18}N_2O_4S$	322.38	90.23	160	2.73	0.86
F	$C_{16}H_{21}N_3O_2S$	319.42	96.5	85	3.01	0.68
G	$C_{12}H_{14}N_2O_3S$	266.32	97.12	158	2.17	0.75
H	$C_{14}H_{16}N_2O_3S$	292.36	82.15	170	2.85	0.8
I	$C_{14}H_{16}N_2O_2S$	276.36	85.36	110	2.91	0.58

Table 1.2. Preliminary QSAR Study

Compound Code	Log P	Tpsa	N Atoms	Non	Nohnh	N Violations	N Rotb	Volume
A	3.59	50.36	20	4	2	0	4	261.54
B	3.57	50.36	20	4	2	0	4	261.54
C	3.54	50.36	20	4	2	0	4	261.54
D	2.82	96.18	22	7	2	0	5	271.34
E	2.25	79.82	22	6	3	0	5	281.57
F	3.01	53.60	22	5	2	0	5	293.91
G	2.17	63.50	18	5	2	0	4	227.57

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H	2.85	70.59	20	5	3	0	4	256.02
I	2.91	50.36	19	4	2	0	4	248.00

Table 1.3. Drug Likeness by Molinspiration

Compound Code	Gpcr Ligand	Ion Channel Modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
A	-0.99	-0.55	-1.57	-0.91	-1.49	-0.91
B	-0.96	-0.5	-1.46	-0.95	-1.41	-0.85
C	-0.97	-0.51	-1.44	-0.94	-1.41	-0.85
D	-0.97	-0.47	-1.52	-0.85	-1.37	-0.88
E	-0.84	-0.51	-1.23	-0.72	-1.26	-0.73
F	-0.81	-0.49	-1.19	-0.76	-1.20	-0.76
G	-1.37	-0.86	-2.08	-1.43	-1.91	-1.17
H	-9.7	-0.48	-1.44	-0.80	-1.37	-0.77
I	-1.05	-0.53	-1.54	-1.01	-1.48	-0.87

Table 1.4. Molecular Interactions of Ligand Compounds with Protein in 1jil

Compound Code	H-Bond Interaction	H-Bond Distance (Å)	Non-Bonding Interactions	Binding Energies (Kcal/ Mole)
A	UNK N : VAL 224 :A	2.21	Ser 194, Leu 223, Phe 232, Asp 195, Gly 193, Leu 52, Pro 32	-6.83
B	UNK O : GLY 38: A	1.982	Tyr 170, Asp 177, Gln 174, Asp 80, Thr 75, Gln 196, Gln 190,Val 191, Gln 191, Asn 124	**-8.30**
C	-	-	Arg 227, Pro 336, Pro 326, Ser 382, Glu 381, Ile 338, Asp 384	**-8.28**
D	UNK O : ARG 65 : A	2.065	Ala 335, Pro 336, Ser 330, Leu 329, Asp 384, Phen 92, Arg 227	-6.84
E	UNK O: LYS 90 : A	2.675	Leu 329, Ser 330, Thr 332, Ile 338, Ser 330, Glu 381, Asp 384, Phen 92	-6.81
F	-	-	Pro 326, Ile 338, Ala 335, Pro 336, Asp 384, Arg 227, Arg 65	-6.20
G	-	-	Pro 326, Ile 338, Asp 384, Glu 381, Ala 335, Leu 329, Pro 336, Ser 330	-5.60
H	UNK O : SER 194: A UNK O: VAL 224 : A	2.380 1.796	Leu 329, Pro 326, Ser 382, Glu 381, Asp 384, Ile 338, Thr 332, Ala 335, Arg 227	-6.20
I	UNK O : ARG 227: B	2.152	Phen 92, Lys 90, Asp 384, Tyr 402, Val 109, Leu 108, Ala 409	-6.73
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**

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Table 1.5. Energy Minimization Table

S No	Compound Code	Binding Energy (Kcal/Mol)	Ligand Efficiency	Inhibitory Constant (μM)	Vdw - Hb Desolvation Energy (K Cal/Mol)
1	A	-6.83	-0.34	9.92	-8.02
2	B	-8.30	-0.42	82.14	-8.87
3	C	-8.28	-0.32	20.83	-6.86
4	D	-6.84	-0.32	7.79	-7.54
5	E	-6.81	-0.27	38.83	-7.45
6	F	-6.20	-0.27	38.89	-7.51
7	G	-5.60	-0.29	152.96	-6.24
8	H	-6.20	-0.3	40.96	-7.09
9	I	-6.73	-0.29	106.71	-5.97
10	Ciprofloxacin	-7.32	-0.22	36.49	-8.04

RNA ligase synthetase (PDB ID: 1JIL) with designed potential inhibitors was carried out by using Auto dock 4.2. And the results has been tabulated in Table 1.4. 2D and 3D snapshots depicting the docking poses along with molecular level interactions responsible for the binding have been shown in Figs 1.13, 1.14, 1.15. for the standard control drug Ciprofloxacin and nine novel dihydropyrimidinthione derivatives respectively. Molecular docking study were performed for the synthesized compounds using AUTODOCK software version 1.5.4.

- Antimicrobial Activity - compound B showed the good binding affinitiy (-8.87 kcal/mol) towards the target Tyrosyl t-RNA Ligase Synthetase (1JIL) compared to standard ciprofloxacin (-8.04) respectively.
- Docking scores or binding energies for all the synthesized compounds were shown in the Table.1.4
- Energy minimization values for the obtaine compounds were also depicted in the Table 1.5

***Evaluation of Pharmacokinetics, Drug Likeness and Medicinal Chemistry Friendliness of Molecules–Swiss Adme*:** To be effective as a potent drug, a molecule must reach its target in the body in sufficient concentration, and stay there in a bioactive form long enough for the expected biologic events to occur. Drug development involves assessment of absorption, distribution, metabolism and excretion (ADME) increasingly earlier in the discovery process, at a stage when considered compounds are numerous but access to the physical samples is limited. In that context, computer models constitute valid alternatives to experiments. The Swiss ADME web tool that gives easy efficient input, free access to a pool of fast yet robust predictive models for physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry friendliness, among which in-house proficient methods such as the BOILED Egg, iLOGP and Bioavailability Radar to support drug discovery endeavors were tabulated as **Table 1.7.** Class: <10 – Insoluble, 10 – Poorly, 6- Moderetly, 4 – soluble, 2 – very, 0 highly. During the time- and resource-consuming processes of drug discovery and development, a large number of molecular structures are evaluated according to very diverse parameters in order to steer the selection of which chemicals to synthesize, test and

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promote, with the final goal to identify those with the best chance to become an effective medicine for the patients. The molecules must show high biological activity together with low toxicity. Equally important is the access to and concentration at the therapeutic target in the organism. It has been demonstrated that early estimation of ADME in the discovery phase reduces drastically the fraction of pharmacokinetics-related failure in the clinical phases 1. As per the Swiss ADME predictions, results of which were tabulated in **Tables 1.6, 1.7 and 1.8**; all the synthesized dihydropyrimidinthione derivatives are as per Lipinski's rule. Hence all the synthesized compounds have potential drug likeness, lead likeness, skin permeation and synthetic accessibility. The results showed that the synthesized compounds are polar with good to moderate water solubility and are therefore expected to have good oral absorption and bioavailability. The predicted gastro-intestinal absorption was displayed high and could assess the absence of toxicity at CNS level due to nonpermeation across the BBB. The log p values of all designed compounds were found to be optimal and hence are predicted to have good permeability and oral absorption.

***Elementary Analysis*:** All the synthesized compounds were dertermined for elementary analysis using CHEMSKETCH SOFTWARE. The Elementary values are depicted in the tabular column 1.9.

***Toxicity Assessment*:** All the synthesized compounds were studied for its toxicity profile using OSIRIS PROPERTY EXPLORER software. The results showed that the most of the studied compounds were found

Table 1.7. Drug Likeness Properties using SWISS ADME

Compound Code	Lipophilicity (Log $P_{o/w}$)					Water Solubility (Log S)		
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	ESOL	Ali	SILICOS-IT
A	2.96	2.61	1.61	2.03	3.83	-3.37	-3.99	-4.91
B	2.92	2.61	1.61	2.03	3.83	-3.37	-3.99	-4.91
C	2.87	2.61	1.61	2.03	3.83	-3.37	-3.99	-4.91
D	2.12	1.81	0.86	0.54	1.04	-2.84	-4.12	-3.67
E	2.74	1.60	0.67	0.66	2.77	-2.72	-3.56	-3.84
F	2.90	2.10	1.02	1.45	2.86	-3.02	-3.53	-4.40
G	2.53	1.06	0.55	0.18	2.58	-2.11	-2.68	-3.53

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H	2.32	1.62	0.66	0.95	2.71	-2.63	-3.39	-3.73
I	2.70	1.98	0.96	1.51	3.19	-2.77	-3.34	-4.31

Table 1.8. *In Silico* ADME Prediction using SWISS ADME

Compound Code	GI Absorption	BBB Permeation	P-GP Substrate	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP3A4 Inhibitor	Skin Permeation cm/s
A	High	No	No	Yes	Yes	Yes	-6.34
B	High	No	No	Yes	Yes	Yes	-6.34
C	High	No	No	Yes	Yes	Yes	-6.34
D	High	No	No	Yes	No	No	-6.98
E	High	No	Yes	No	Yes	No	-7.13
F	High	No	No	No	Yes	No	-6.76
G	High	No	No	Yes	Yes	No	-7.16
H	High	No	No	No	Yes	No	-6.93
I	High	No	No	Yes	Yes	No	-6.58

Table 1.9. Elementary Analysis

Compound Code	Molecular Formula	Molecular Weight	Composition
A	C14H12O2N2SCl	310.81	C (54.10%) H (4.86%) Cl (11.41%) N (9.01%) O (10.30%) S (10.32%)
B	C14H12O2N2SCl	310.81	C (54.10%) H (4.86%) Cl (11.41%) N (9.01%) O (10.30%) S (10.32%)
C	C14H12O2N2SCl	310.81	C (54.10%) H (4.86%) Cl (11.41%) N (9.01%) O (10.30%) S (10.32%)
D	C14H12O4N3S	321.36	C (52.33%) H (4.70%) N (13.08%) O (19.92%) S (9.98%)
E	C15H18N2O4S	322.38	C (55.88%) H (5.63%) N (8.69%) O (19.85%) S (9.95%)
F	C16H21N3O2S	319.42	C (60.16%) H (6.63%) N (13.16%) O (10.02%) S (10.04%)
G	C12H14N2O3S	266.32	C (54.12%) H (5.30%) N (10.52%) O (18.02%) S (12.04%)
H	C14H16N2O3S	292.36	C (57.52%) H (5.52%) N (9.58%) O (16.42%) S (10.97%)
I	C14H16N2O2S	276.36	C (60.85%) H (5.84%) N (10.14%) O (11.58%) S (11.60%)

Table.2.0: Osiris Property Explorer – Toxicity Profile

Compound Code	Mutagenic Effect	Tumorogenic Effect	Irritant Effect	Reproductive Effect

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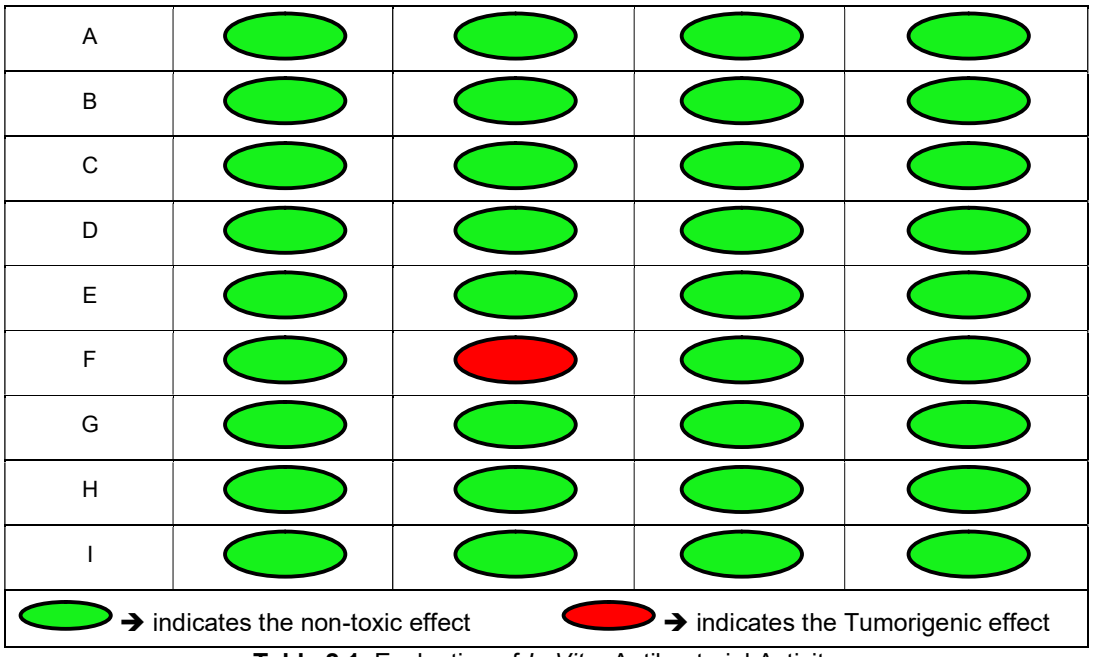

Table 2.1. Evaluation of *In Vitro* Antibacterial Activity

Compound Code	Zone of inhibition			
	Escherichia Coli MTCC 433	*Pseudomonas Aeruginosa MTCC 1934*	*Bacillus Subtilis MTCC 121*	*Methicillin Resistant-Staphylococcus Aureus MRSA*
A	12 mm	15 mm	11 mm	11 mm
B	15 mm	17 mm	14 mm	13 mm
C	17 mm	19 mm	15 mm	14 mm
D	15 mm	18 mm	12 mm	-
E	16 mm	11 mm	11 mm	-
F	11 mm	12 mm	12 mm	13 mm
G	12 mm	13 mm	12 mm	-
H	12 mm	13 mm	11 mm	-
I	13 mm	12 mm	12 mm	-
Ciprofloxacin	22 mm	21 mm	20 mm	-

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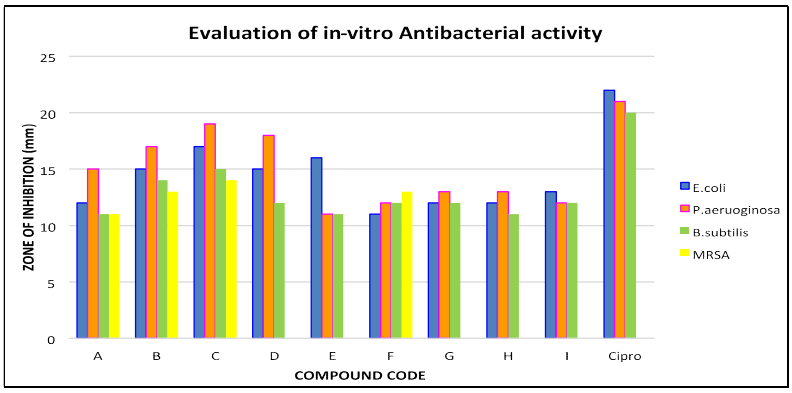

Fig.2.9

to be non-toxic except compound F which showed Tumorogenic effect. The risk of toxicity profile for the predicted compounds were depicted in the Table 2.0.

Evaluation of In Vitro Anti-Bacterial Activity: All the synthesized compounds were screened 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 was shown in the Table 2.1. Compound C showed significant antibacterial activity against *E.coli* ***MTCC 433*** (17mm), *Pseudomonas aeruginosa* ***MTCC 1934*** (19mm), *Bacillus subtilis* ***MTCC 121*** (15mm) and *MRSA (*14mm) respectively.

Among the tested compounds, compound C produces a very good antibacterial activity. Compound A, B, C and F showed the good antibacterial activity against MRSA compared to std ciprofloxacin.

The Schematic representation for the evaluation of *In vitro* antibacterial activity were depicted in the Fig.2.9

Discussion

Antibiotic resistance is all the time more recognized as a serious and permanent public health concern and is usually considered to be a consequence of wide use and misuse of antibiotics. So, the drug resistivity of microbes has been increased enormously[2]. To combat this antimicrobial resistance, it is necessary to develop a new and effective antimicrobial drug[3]. So, we sought to design, synthesis of novel dihydropyrimidinthione is one of the promising candidates attracted substantial attention of the medicinal chemists[3]. Dihydropyrimidinthione[20] were reported as therapeutic leads to develop newer and effective pharmacophores with enhanced and a versatile range of medicinal activities like such as antiviral[5], anticancer[3], antibacterial[3], antituberculosis[4], antihypertensive[6], antiarrythmic activities[7].

Most of the drugs on the market today are entirely chemically synthesized in the laboratory. Several medicinal chemists had synthesized dihydropyrimidine (DHPMs) derivatives showing a wide spectrum of therapeutic actions as antibacterials, antivirals as well as antitumor agents.

Green Chemistry approach[32] shows synthesis is in good yields and in less time and also avoids problems associated with solvent and reagents use. It was found that PTSA (p-toluene sulphonic acid) works as an excellent catalyst for the one-pot three components and solvent free synthesis of dihydropyrimidinthione. This technique is superior to the existing methods. Since grinding does not require solvents leading to a

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safe and environmental friendly synthesis solvent free approach opens up numerous possibilities for conducting rapid organic synthesis and functional group transformations more efficiently[33]. Additionally there are distinct advantages of these solvent free reactions. It prevents pollution in organic synthesis at source[33].In solvent free organic reactions reagents react together in the absence of any solvent have been reviewed as a fast developing technology. It is required to develop safe, practical and environmental friendly process. Many exothermic reactions can be accomplished in high yield by using a technique known as "**Grindstone chemistry** "which is one of the **"Green Chemistry Technique"**[34].

In the conventional synthesis of dihydropyrimidinthione derivatives requires nearly 20 hours to complete the synthesis without using any catalyst and green chemistry approach. Comparison between conventional[27] and solvent free synthesis[30] was done by comparing total reaction time and percentage yield. The results suggest that solvent free synthesis[15] lead to higher yields within very short reaction times. In keeping in view, the biological significance, medicinal utility and inorder to minimize of solvent and also time consuming with good yield product of dihydropyrimidinthione derivative[25], the present study involved solvent free synthesis[11], molecular docking and study of some novel dihydropyrimidinthione derivatives[13] against Tyrosyl t-RNA Ligase Synthetase as a target enzyme. solvent-free conditions with catalyst p-TPSA [31] (scheme II) or without catalyst (scheme I) to afford the corresponding dihydropyrimidinthione in good yield (76–96%).

Conclusion

Novel dihydropyrimidinthione derivatives were designed and synthesized using Green chemistry approach, eco-friendly technique in order to increase the speed of the reaction and the percentage yield. The purity of the synthesized compounds was determined by TLC and melting point determination. The structures were characterized using predicted spectrum from CHEMSKETCH, CHEM DRAW and MARVIN SKETCH software. Drug likeness properties were studied using MOLINSPIRATION software. All the synthesized compounds obey's the Lipinski's rule of five. All the synthesized compounds were studied for its toxicity profile using OSIRIS property explorer software. The results showed that the most of the studied compounds was non-toxic, except compound F which shows tumorogenic effect. All the synthesized compounds showed good binding energies against the target enzyme for antimicrobial activity.Compound B showed significant antibacterial activity compared to Standardciprofloxacin at the concentration of 100µg/ml. This result showed Compound B may be suitable newer molecule for further development of potential antimicrobial agent for emerging diseases.

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