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Research Article

Design, Synthesis and Antimicrobial Activities Evaluation of 1, 3 Thiazine Derivatives

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Abstract

Some novel 30 derivatives of 1, 3 thiazine have been designed for Computational studies. This derivatives of 1,3 thiazine were subjected to molecular docking studies against *E. coli* Glucosamine -6 P Synthase in Complex with Fructose -6 P (PDB ID- 4 AMV) and Crystal structure of Peptide deformylase from *Staphylococcus Aureus* Complex with Actinonin (PDB ID- 1Q1Y) using Molegro Virtual Docker software. On the basis of docking results 10 derivatives of thiazine were selected and synthesized by the condensation of 2-hydroxy-3- nitro5-chlorochalcones with thiourea and phenylthiourea in ethanol containing aqueous KOH solution. All synthesized compound have been screened for in-vitro evaluation of antimicrobial activities by agar plate techniques. The results indicated that all the synthesized 1, 3 thiazine derivatives shows considerable antimicrobial activities. This study suggested that 1, 3-thiazine derivatives founded to have potent antimicrobial activity against the selected pathogenic organisms and posses more antimicrobial activities on gram negative *(E. coli)* bacteria than gram positive bacteria *(S. aureus)*. Keywords: 1, 3 thiazine, molecular docking, analysis, characterization, antimicrobial activities, thiourea, phenyl thiourea, gram negative bacteria.

Keywords: 1, 3 Thiazine; Molecular Docking; Analysis; Characterization; Antimicrobial Activities; Thiourea; Phenyl Thiourea; Gram Negative Bacteria

Introduction

In medicinal chemistry, pharmacology and biotechnology, the process by which drugs are discovered and designed is known as Drug Discovery. The process of drug discovery involves the identification of candidates, synthesis, characterization, screening and assays for therapeutic efficiency.

The drug discovery process increased in intensity because of the major screening and chemical synthetic effort in the pharmaceutical industry in worldwide industrialized countries. Despite understanding biological system and advance in technology, the drug discovery is still a long process with low rate of new therapeutic discovery. [1].

Steps of drug discovery

The drug discovery involves following steps:

- Screening of new compound
- Lead optimization
- Natural compounds mimicking
- Clinical development.

Organic chemistry play an important role in modern science and has wide varieties applications in different fields since many research has been going on to synthesize new organic compounds and derivatives of naturally occurring ones.

Heterocyclic chemistry research encompasses almost half of the organic chemistry research throughout the world. A huge amount of bioactive organic compounds that contain heterocyclic frameworks play a vital part in the medicinal field.

It is commonly reported that heterocycles having sulphur or nitrogen atoms or both of them are the general features present in the structures of most of the pharmaceutical and natural compounds [2,3]. They also act as multidentate ligands for different metals due to the presence of nitrogen and sulfur atoms and are thus used extensively in coordination chemistry to obtain new frameworks with potential bioactivity [3]. According to Joshi., *et al*. it has also been identified that several heterocyclic compounds in the developmental phase have the potential to be part of new drugs and also play an important role in modern drug discovery [4].

Antimicrobial agents

Antimicrobial Agents are agents that kill microorganisms or stop their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungals are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called biostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis [5,6].

Type of antimicrobial drugs

- Antibacterial drugs
- Antifungal drugs
- Antiviral drugs
- Anti-helminth drugs.

Antibacterial agents

The discovery of antibiotics has been regarded as one of the most significant medical achievements of the twentieth century. Antibiotics have saved millions of lives and enabled important medical procedures, including surgery and cancer chemotherapy. The emergence and spread of antibacterial resistance in all geographical areas; including bacteria that cause hospital and community-acquired infections is however jeopardizing the effectiveness of these potentially life-saving treatments. The threat includes the spread of multidrug-resistant bacteria and infections with no therapeutic options have been reported. The rise in resistance not only impedes the ability to treat bacterial infections in humans and animals but has broader societal and economic effects that ultimately threaten achievement of the Sustainable Development Goals. This situation requires urgent, coordinated action at global, regional and national levels. Resistance is a natural phenomenon, and it is inevitable that it will develop to all antibiotics at some time. As misuse and overuse of antibiotics accelerate the development of resistance, antibiotics should be used more responsibly and new antibacterial treatments should be developed to counteract emerging resistance. However, there are challenges which are both; 'scientific' for the discovery of new antibiotics, and 'economic' for ensuring investment into research and development [7,8].

Most of the new antibiotic classes; however, target Gram-positive bacteria, while the major challenge is to find new antibiotics against Gram-negative bacteria. These are identified as a critical priority by WHO on its priority pathogens list for R&D of new antibiotics. Because of the complexity of the Gram-negative cell wall, discovery of novel antibiotics that can permeate this barrier and stay inside the bacterium is very challenging. The lack of new, quality lead chemicals to test against Gram-negative bacteria is another major impediment to discovery. Recently, drug resistance due to the extensive abuse and over-use of antibiotics has become an increasingly serious problem, making the development of alternative antibiotics a very urgent issue [9].

Thiazine

Thiazine is an organic compound containing a ring of four carbon, one nitrogen and one sulfur atom. There are three isomers of thiazine, 1, 2-thiazine, 1, 3-thiazine, and 1,4-thiazine, which differ by the arrangement of the nitrogen and sulfur atoms in the ring. They arefound to be fairly stable. Thiourea has been used in the synthesis of heterocyclic rings containing nitrogen and sulphur.

Structure of thiazine

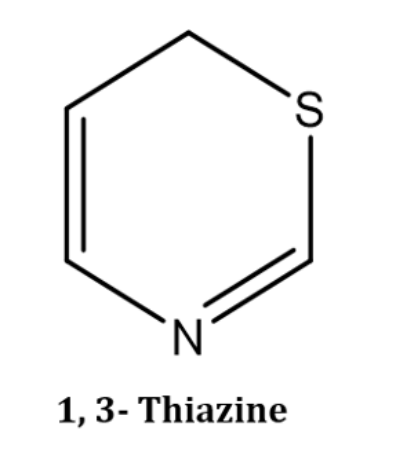


Figure a

The thiazines possess a nitrogen and sulphur atoms in a six member ring (Figure), that is believed to be important for their antifungal, anticonvulsant, and antiviral activities. The uniqueness and resourcefulness of the simple thiazine chemical structure and easy availability make thiazines and their derivatives amongst the most gifted sources of bioactive compounds. Thiazine is a heterocyclic compound having one nitrogen and sulphur atom at varied positions in the six membered ring exist as 1,2; 1,3; 1,4-thiazines and subsequently their derivatives [10].

Figure b

Biological activities of thiazine and benzothiazine derivatives

Heterocyclic thiazine and benzothiazine compounds are a crucial part of medicinal organic chemistry due to their useful medicinal properties. The largely unexplored heterocyclic compounds like thiazines possess a variety of pharmacological activities, ranging from antitumor, antipsychotic and anti-inflammatory properties on the one hand, while on the pathogenic side, they are equally important due to their antibacterial, antitubercular, antifungal, antiviral and antiprotozoal activities.

Thus, thiazine compounds display a wide range of beneficial properties [11]. Antimicrobial Activities Phenothiazines and their novel derivatives exhibit valuable biological activities both *in vivo* and *in vitro*. These compounds show good results against various diseases caused by bacteria, viruses, fungi, mollusca, or protozoa. The activities of these compounds were examined by applying them on various organisms such as mammals infected with pathogenic bacteria and viruses, cell lines, etc. [12]. The 1,3-thiazine moiety is the functional part of cephalosporins that are the β-lactam antibiotics, active against most Gram positive and a number of Gram negative pathogenic bacteria. Cephalosporins have a similar mode of action like penicillins and have an identical β-lactam ring, however, there are more atoms at the side rings [13]. Most of the penicillin-resistant bacteria are sensitive to cephalosporins, although there are some exceptions [13]. Bacterial resistance is a common problem to cephalosporins, for example the enteric bacteria are resistant to almost all of the third and fourth generation drugs [14]. There are several strains of *Staphylococcus aureus* that are even resistant to the fifth generation cephalosporins like ceftobiprole and ceftaroline. Therefore, novel strategies are being devised to generate future generation cephalosporins [14]. In addition to broad spectrum activities, 1,3-thiazine and its derivatives contains wonderful properties like antitumor, insecticide, and fungicidal. Further, these can be used as anti-radiation agents in radiation-sickness [15]. The 4-hydroxy- N0-(benzylidene)-2H-benzo(e)(1,2) thiazine-3-carbohydrazide 1,1-dioxides [16] compounds possess not only antibacterial but also has radical scavenging activities and these properties are raised when more lipophilic is the compound [17]. Thus, such thiazine derivatives are useful chemical tools in biochemistry research and can be used in respiratory and photosynthetic electron transfer research where the free radicals are produced during normal activity as well as malfunction of some pathways. Patel., *et al*. made around forty different 1,2-benzothiazine derivatives, among which some only exhibited antibacterial activity against Gram positive bacteria [18].

1, 3 thiazine

Structure of 1, 3thiazines possesses an N-C-S linkage that is believed to be very useful units in the fields of medicinal and pharma-

ceutical chemistry [10] 1,3thiazines and its derivatives have been reported to exhibit a variety of biological activities like Antibacterial, Antifungal, Antitubercular [19], Anti-inflammatory [20], Analgesic, Sedative-hypnotic, Immunosuppressive agents, etc. Some derivatives of thiazine are cannabinoid receptor agonists, also they can act as an anti-hypotensive. Moreover, thiazine derivatives can be used for gastrointestinal disorders or diabetes prevention. Condensed heterocyclic systems possessing thiazine ring have been reported as antioxidants, and calcium channel modulators 1, 3-thiazines.are of great importance because they form part of the framework of cephalosporins (3, 6-dihydro-2H,1,3-thiazine) and also in some other medicinally important compounds like Xylazin (agonist at the α2 class of adrenergic receptor is used for sedation, anesthesia, muscle relaxation, and analgesia in animals), Chlormezanone (used as an anxiolytic and a muscle relaxant) etc. [9].

Biological potential of 1, 3-thiazines

Antimicrobial activity

1,3-Thiazines and their derivatives have significant antimicrobial potential against various strains of bacteria, fungi etc. The core moiety of 1,3-thiazines (C-N-S) forms an active site in antibiotics like Cephalosporins. 1,3-Thiazines derived from chalcones viz. 4-(2-hydroxy-3,5-dichlorophenyl)-6- (ethyl)-2-iminophenyl-3phenyl-1,3-thiazine, 4-(2-hydroxy-3,5-dichlorophenyl)-6-(ethyl)-2- iminophenyl-1,3-thiazine etc. have also been evaluated for their *in vitro* antimicrobial activity against various gram positive- *Streptococcus aureus*, *S. subtilus* and gram negative bacteria- *E. coli* and *P. aeruginosa*. Mamoru Koketsu., *et al.* 2002 synthesized series of 5,6- dihydro-4H-1,3-thiazine derivatives [22] which showed antimicrobial activity against *M. tuberculosis H37Rv*. Tarik EL-Sayed Ali., *et al.* 2010 synthesized 1,3-thiazine derivatives having acridine ring which besides showing antimicrobial activity against above mentioned species, also exhibit antibacterial activity against *Streptococcus pyogenes* and *Pseudomonas fluorescens* and *Pseudomonas phaseolicola* and antifungal activity against *Fusarium oxysporum* and *Aspergillus fumigates.* Ramesh L. Sawant., *et al.* 2011 introduced electron donating groups like hydroxyl and methoxy group at the fourth position of phenyl rings in the series of 6-[4-substitutephenyl]-4-phenyl-6H-1,3-thiazine-2-amines and N-[6-(4- substitutedphenyl)-4-phenyl-6H-1,3-thiazine-yl] acetamides which enhances their antimicrobial activity. Farooque Haider Zulfequar Haider, 2012 synthesized series of 4-(2-hydroxy-5-substitutedphenyl)-5-benzoyl-6-substitutedphenyl-2-imino-6H-2,3-dihydro-1,3-thiazine derivatives which exhibits its antimicrobial activity due to the presence of phenolic group. Its antibacterial activity has been observed to be enhanced by increasing the number of heteroatoms in the heterocyclic system. Thanusu J., *et al.* 2010 introduced morpholine ring in the series of 4-(4-morpholinophenyl)-6-aryl-1,3- thiazin-2-amines which showed substiantial antibacterial activity against *V.cholera* etc. and antifungal activity against various strains of fungi viz. *Rhizopus, M. gyseum* [23] etc.

Other activities of 1, 3-thiazines

Li Fu., *et al.* 2010 procured series of 6H-2-amino-4-aryl-6-(4-β-D-allopyranosyloxyphenyl)-1,3-thiazines by Claisen Schmidst condensation which show strong calming activity in comparison with parent helicid.4 T.P.Trofimova., *et al.* 2008 gave a reaction scheme to synthesize 2-N-acylamino-5,6-dihydro-4H- 1,3-thiazines which showed excellent NOS inhibiting activity both *in vivo* and *in vitro* and also act as antihypotensive agents *in vivo*. 16 Kai H., *et al.* 2008 synthesized 2-arylimino-5,6-dihydro-4H-1,3- thiazines which show profound analgesic properties [24]. Tetrahydro-1,3-thiazines derivatives, tetrahydro [1, 3]-thiazine-4-one-6-carboxylic acid, tetrahydro [1,3]-thiazin-4,6-dione derivatives, 2-(2-amino-4-phenyl-6H-1,3-thiazin-6-yl)-4-[3-(2-amino-4-phenyl-6H-1,3-thiazine-6-yl) 4-hydroxy-benzyl]phenol and 2-[2-amino-4-(4-chlorophenyl)-6H-1,3-thiazin-6-yl]-4-hydroxybenzyl}phenol etc. have also been known to exhibit strong anti-inflammatory activity and most of them are immunotropic in nature [Zawisza T., *et al.* (1978&1981); R. Kalirajan., *et al.* (2009), A. Nagaraj., *et al.* (2008)]. Derivatives of 1,2,4-triazolo [3, 2-b]-1,3-thiazine-7-ones and amino/ guanidine thiazine derivatives besides, possessing anti-inflammatory activity, also exhibits analgesic properties [Tozkoparan B., *et al*. (2002); Vijay V. Dabholkar., *et al*. (2011)] [25]. The derivatives of 1H-pyrrolo [1, 2-c] [1,3] thiazine have been reported to show moderate anticonvulsant activity [Tadeusz S. Jagodzinski., *et al.* 2003] [26].

Agrochemical uses of 1,3-thiazine derivatives

Tetrahydro-2-(nitromethylene)-2H-1,3-thiazine (Nitromethylene) possess strong insecticidal properties (111) [Margulies, L., *et al*. (1988)] [27]. Perhydro dervivatives of 1,3-thiazine have obtained patent for their insecticidal properties against various nematodes [Jean-dominique bourzat., *et al*. (1981)] [28].

Thus, variously substituted 1, 3-thiazine derivatives procured largely through cyclo-condensations and few ring transformations have great synthetic utility, particularly for the synthesis of different heterocyclic systems. Besides having synthetic applications, these have also been remarkably known for their biological activities viz. pharmaceutical, agrochemical etc.

Research envisaged

Antibacterial agents are used for the treatment of bacterial diseases by interfering with the growth and reproduction of bacteria. Generally heat, chemicals and other antibiotic drugs having antibacterial properties were used for the treatment purpose in past.

Globally, antibacterial resistance has now a day seems very big challenge to the effective treatment of infections. Drug resistance adversely affects both sides: clinical as well as financial therapeutic outcomes. By seeing this scenario, new antibacterial agent having potent activity against the resistant microorganisms needed to be developed. Although many effective antibiotics are available now a day, but the change in nature of health cares and constant evolution of bacterial pathogens will create need and opportunities for significantly improved drugs.

Thus in order to overcome these problems there is a need to develop alternate therapeutic agents with selective antibacterial activity without risk of harmful side effects and resistance.

The thiazines possess a nitrogen and sulphur atoms in a six member ring that is believed to be important for their antifungal, anticonvulsant, and antiviral activities. The uniqueness and resourcefulness of the simple thiazine chemical structure and easy availability make thiazines and their derivatives amongst the most gifted sources of bioactive compounds.

Literature survey shows, nucleus has diverse antibacterial activity. Therefore, keeping the above consideration it was thought worthwhile to design a new series of thiazine derivatives with different substituents, on position 2,3and 4 of phenyl ring attached at 6 position of thiazine ring with different substituted benzaldehyde derivatives as electron-withdrawing group, and evaluate them for antibacterial activity.

Plan of work

- Literature survey.

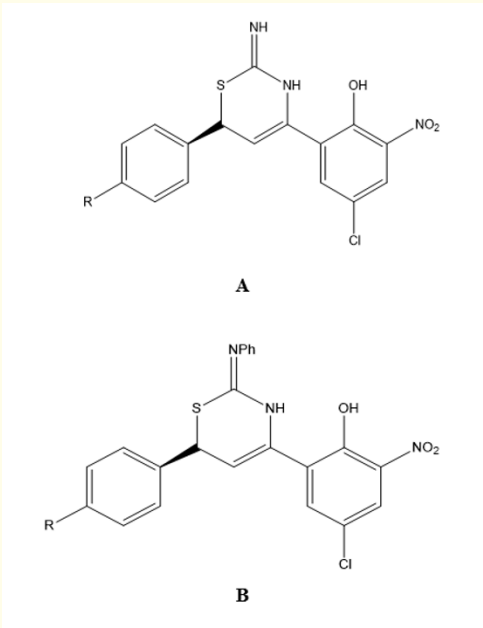


Figure c: General Structure.

- Selection of compounds for synthesis.
- Optimization of reaction conditions.
- Synthesis of designed compounds.
- Determination of physicochemical properties-
  - Solubility.
  - Melting Point determination.
  - R_f value determination.
  - Partition coefficient (Log P determination).
- Microbiological evaluation of synthesized compounds-
- Minimum Inhibitory Concentration.

Materials and Methods

Computational studies

Computational studies were used to designed the series of 1, 3- Thiazine derivatives.

Docking

Docking is a computerized method through which we can be determines the binding efficiency of a compound with the active

site of the proteins. This method determines the orientation of the compounds, conformational geometry of the compound and scoring.

Steps involved in docking

Proteins download

The "PDB ID-1Q1Y"is the PDB ID for *Staphylococcus aureus*, and "PDB ID-4AMV"is the PDB ID for *Escherichia coli.* These protein files were downloaded from RCSB (research Collaboratory for structure bioinformatics) protein data bank, and saved in PDB format.

Figure 1: Structure of 4AMV (PDB ID).

Protein preparation

Proteins were prepared in UCSF Chimera 1.7S Software by, selecting in suitable chain. Water molecules were removed, and the protein was saved in PDB format.

Figure 2: Structure of 1Q1Y (PDB ID).

Ligand preparation

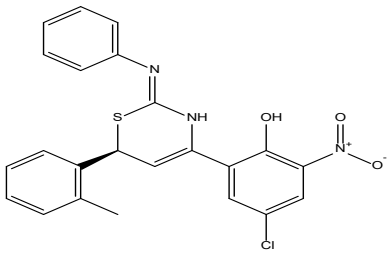
The structure of ligands was drawn by using ChemDraw Ultra 8.0 and energy was minimized in chem Draw 3D Ultra 8.0 and saved in mole2 format.

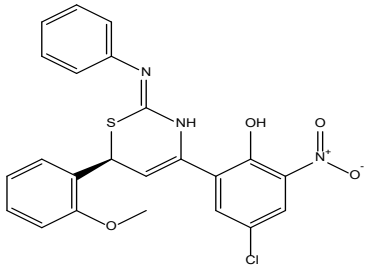
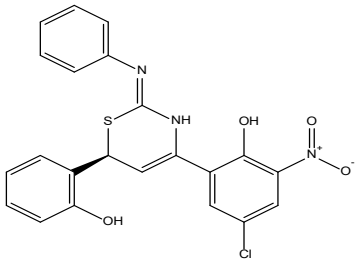
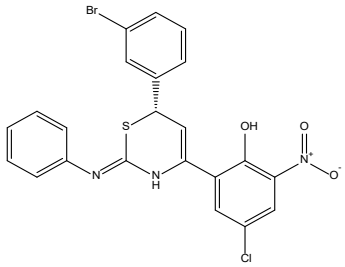
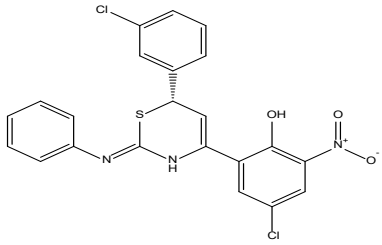
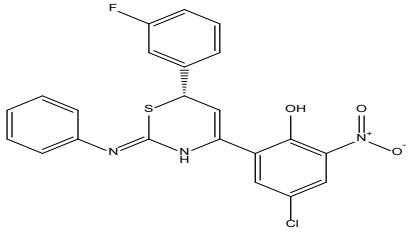
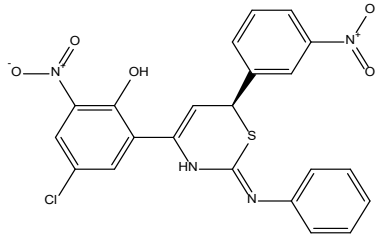
Defining binding site

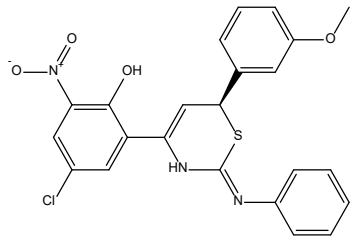
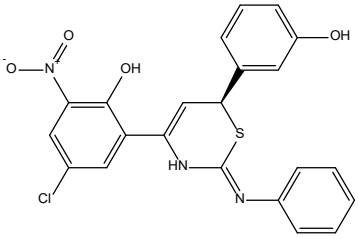
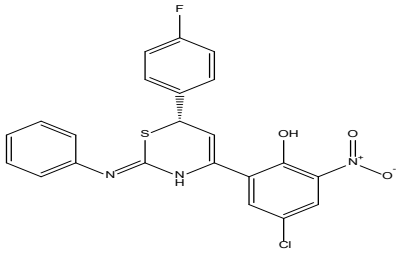
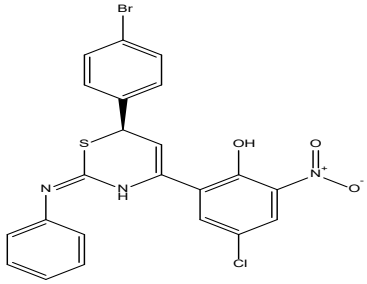
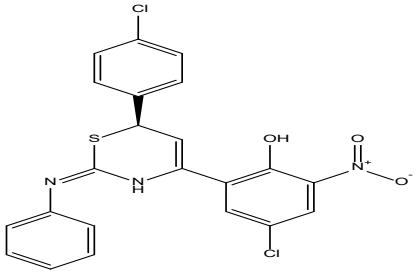
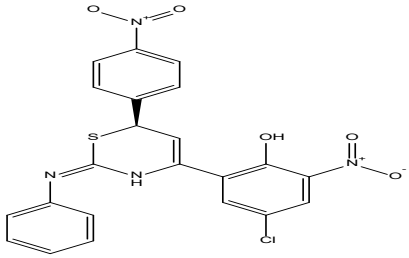
The binding site of protein was defined by selecting all atoms within 10Å, which was found by redocking the native ligand at the active site.

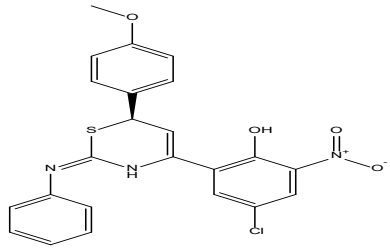
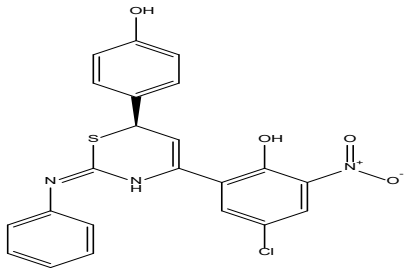
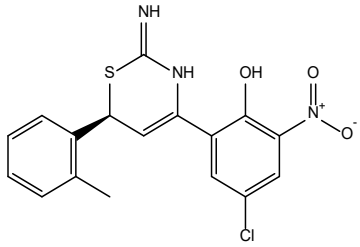
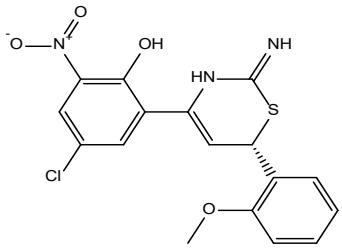
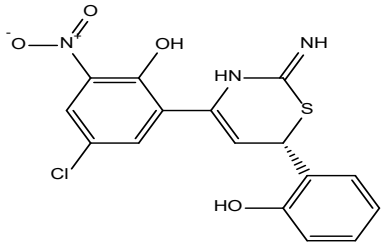
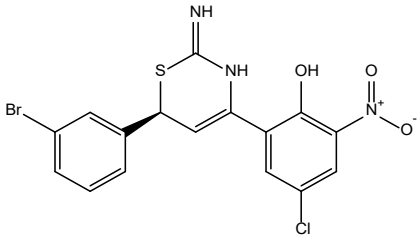
Docking in molegro virtual docker

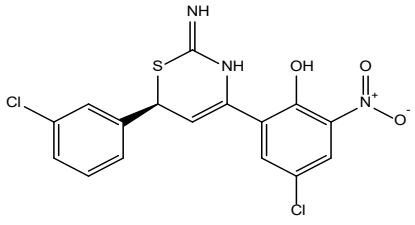
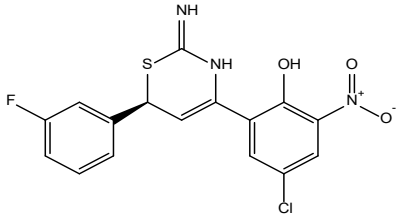
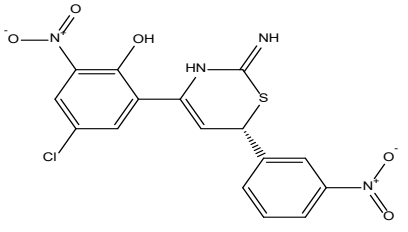
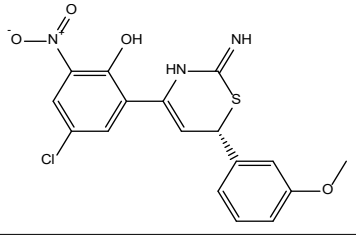
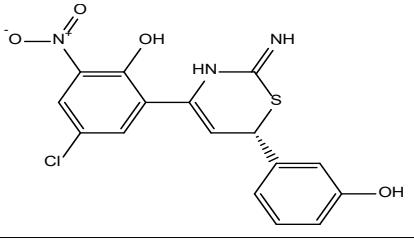
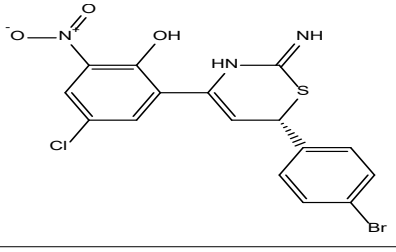
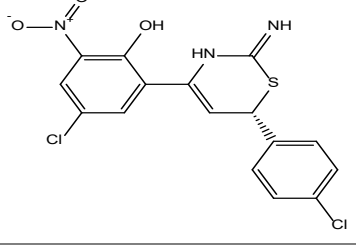
Docking is an essential tool for fast consideration of the binding efficiency of ligand with its target protein i.e. enzyme. Docking is a commercial and timesaving method for the invention of lead compounds. In current years, the virtual screening for docking of small molecules with a known protein structure has become a powerful

Compound code	IUPAC Name	Structure
Ptt2m	(S,Z)-4-chloro-2-nitro-6-(2-(phenylimino)-6-(o-tolyl)-3,6-dihydro-2H-1,3-thiazin-4-yl)phenol	

Ptt2OCH3	(S,Z)-4-chloro-2-(6-(2-methoxyphenyl)-2-(phenylimino)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Ptt2OH	(S,Z)-4-chloro-2-(6-(2-hydroxyphenyl)-2-(phenylimino)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Ptt3Br	(S,Z)-2-(6-(3-bromophenyl)-2-(phenylimino)-3,6-dihydro-2H-1,3-thiazin-4-yl)-4-chloro-6-nitrophenol	
Ptt3Cl	S,Z)-4-chloro-2-(6-(3-chlorophenyl)-2-(phenylimino)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Ptt3F	(S,Z)-4-chloro-2-(6-(3-fluorophenyl)-2-(phenylimino)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Ptt3NO2	(S,Z)-4-chloro-2-nitro-6-(6-(3-nitrophenyl)-2-(phenylimino)-3,6-dihydro-2H-1,3-thiazin-4-yl)phenol	

Ptt3OCH3	(S,Z)-4-chloro-2-(6-(3-methoxyphenyl)-2-(phenylimino)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Ptt3OH	(S,Z)-4-chloro-2-(6-(3-hydroxyphenyl)-2-(phenylimino)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Ptt4F	(S,Z)-4-chloro-2-(6-(4-fluorophenyl)-2-(phenylimino)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Ptt4Br	(R,E)-2-(6-(4-bromophenyl)-2-(phenylimino)-3,6-dihydro-2H-1,3-thiazin-4-yl)-4-chloro-6-nitrophenol	
Ptt4Cl	(R,E)-4-chloro-2-(6-(4-chlorophenyl)-2-(phenylimino)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Ptt4NO2	(R,E)-4-chloro-2-nitro-6-(6-(4-nitrophenyl)-2-(phenylimino)-3,6-dihydro-2H-1,3-thiazin-4-yl)phenol	

Ptt4OCH3	(R,E)-4-chloro-2-(6-(4-methoxyphenyl)-2-(phenylimino)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Ptt4OH	(R,E)-4-chloro-2-(6-(4-hydroxyphenyl)-2-(phenylimino)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Tt2M	(S)-4-chloro-2-(2-imino-6-(o-tolyl)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Tt2OCH3	(S)-4-chloro-2-(2-imino-6-(2-methoxyphenyl)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Tt2OH	(S)-4-chloro-2-(6-(2-hydroxyphenyl)-2-imino-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Tt3Br	(S)-2-(6-(3-bromophenyl)-2-imino-3,6-dihydro-2H-1,3-thiazin-4-yl)-4-chloro-6-nitrophenol	

Tt3Cl	(S)-4-chloro-2-(6-(3-chlorophenyl)-2-imino-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Tt3F	(S)-4-chloro-2-(6-(3-fluorophenyl)-2-imino-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Tt3NO2	(S)-4-chloro-2-(2-imino-6-(3-nitrophenyl)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Tt3OCH3	(S)-4-chloro-2-(2-imino-6-(3-methoxyphenyl)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Tt3OH	(S)-4-chloro-2-(6-(3hydroxyphenyl)-2-imino-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Tt4Br	(S)-2-(6-(4-bromophenyl)-2-imino-3,6-dihydro-2H-1,3-thiazin-4-yl)-4-chloro-6-nitrophenol	
Tt4Cl	(S)-4-chloro-2-(6-(4-chlorophenyl)-2-imino-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	

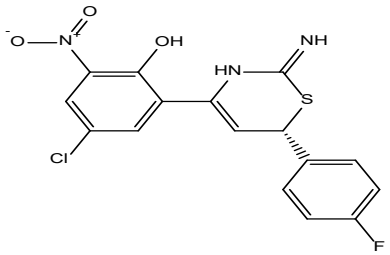
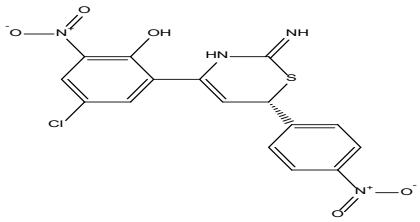
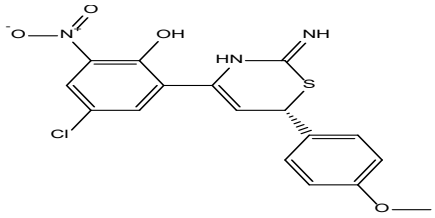
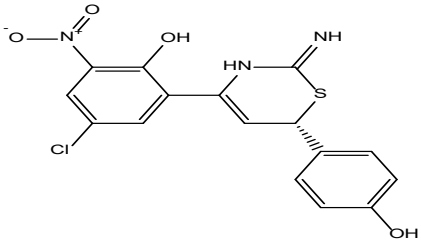
Tt4F	(S)-4-chloro-2-(6-(4-fluorophenyl)-2-imino-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Tt4NO2	(S)-4-chloro-2-(2-imino-6-(4-nitrophenyl)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Tt4OCH3	(S)-4-chloro-2-(2-imino-6-(4-methoxyphenyl)-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	
Tt4OH	(S)-4-chloro-2-(6-(4-hydroxyphenyl)-2-imino-3,6-dihydro-2H-1,3-thiazin-4-yl)-6-nitrophenol	

Table 1: Structure of ligand prepared with compound code ande IUPAC name.

tool for drug design and also become an essential part of the drug discovery. Molegro Virtual Docker is non-commercial docking software. Molegro Virtual Docker 6.0 developed by CLC drug discovery Workbench.

Docking Score of compounds by using Molegro Virtual Docker 6.0 software

Analysis of docking score

- **4AMV:** The entire 30 1, 3 Thiazine derivatives bind with 4AMV protein (Glucosamine fructose 6-p- aminotransferase) and interaction was observed. Ptt4Cl showed the highest MolDock Score -110.723 as compared with reference drug Ampicillin.

Compound Code	MolDock Score	Rerank Score	HBond
Ptt4Cl	-110.723	-78.4613	-5.48575
Tt4OH	-106.654	-81.0678	-12.0967
Tt2OCH3	-105.444	-82.119	-9.53857
Tt4F	-101.536	-84.3331	-8.70252
Ptt3OCH3	-101.001	-73.0568	0
Tt3NO2	-100.075	-83.0619	-6.9303
Tt3OCH3	-99.7293	-81.9545	-5.54038
Ptt3OH	-97.2969	-70.4167	-4.49777
Ptt4F	-96.3454	-53.6497	-4.13648

Tt3OH	-95.1179	-71.0299	-9.45847
Tt4OCH3	-93.706	-76.7804	-3.95941
Ptt4F	-93.3741	-74.005	-5.86158
Ptt2OCH3	-92.5215	-65.9773	0
Tt2M	-91.3958	-75.2571	-3.2033
Tt2OH	-89.3392	-77.6952	-8.23625
TT3F	-89.1138	-74.7265	-5.3147
TT4Br	-88.8064	-74.5592	-6.18152
Ptt4NO2	-88.6181	10.7845	-6.41836
Tt3Br	-88.4113	-64.5513	-6.57065
Tt3Cl	-87.8743	-73.2045	-8.15926
Tt4NO2	-86.8956	-58.2635	-9.75651
Tt4Cl	-86.7749	-67.5634	-6.83585
Ptt4OCH3	-86.7144	19.3956	-6.50447
Ptt2M	-86.1307	-26.7007	-6.73811
Ptt3Br	-85.2379	25.2003	-8.73745
Ptt4Br	-82.4321	19.6867	-6.4191
Ptt4OH	-81.9273	19.5981	-6.47967
Ptt3NO2	-79.8643	72.2411	-6.64596
Ptt2OH	-73.8775	-16.0809	0.881268
Ptt3F	-72.9588	-3.93201	-8.5393
Ptt3Cl	-69.3499	40.2221	-6.63596

Table 2: Docking score of compounds for *E. coli* (4AMV).

Compound Code	MolDock Score	Rerank Score	HBond
Ptt3OCH3	-96.683	-77.2286	-1.35372
Ptt3NO2	-96.5314	-79.9126	-1.58689
Ptt4NO2	-96.5073	-80.1216	-4.02885
Ptt4OCH3	-95.4961	-77.3788	-3.69138
Ptt4OH	-94.7954	-78.3154	-5.3336
Ptt3OH	-94.431	-72.8265	-5.96018
Ptt3F	-92.701	-73.9804	-1.32963
Ptt3Cl	-92.1713	-73.5393	-1.41149
Ptt4Br	-92.1642	-75.5591	-4.89751
Ptt2OCH3	-91.4593	-67.6821	-3.02657
Tt3OCH3	-91.0537	-75.6174	-8.00002
Ptt2M	-90.7249	-72.7707	-1.29751
Ptt2OH	-90.2557	-71.9369	-4.85802
Tt3NO2	-90.2013	-73.9158	-4.97568
Ptt4F	-89.904	1.32502	-2.10633
Ptt3Br	-89.4501	-72.4944	-1.22774
Tt4OCH3	-87.6422	-70.5666	-6.48139
Ptt4Cl	-87.4778	-66.6463	-7.1557
Tt2OCH3	-87.0455	-72.6424	-4.83831
Tt3OH	-86.8145	-70.3878	-10.5391
Tt4NO2	-85.0538	-69.3519	-4.87202
Tt4Br	-84.5862	-66.6038	-4.37323
Tt4F	-84.0047	-66.2733	-5.01028
Tt4Cl	-83.9601	-66.2623	-4.97171
Tt3F	-83.8048	-67.6772	-4.613
Tt2M	-83.3401	-68.0274	-4.71991
Tt4OH	-83.252	-66.2025	-3.71184
Tt3Cl	-83.022	-66.7699	-4.46023
Tt3Br	-82.6209	-66.2686	-4.67272
Tt2OH	-81.2521	-66.3319	-6.93566

Table 3: Docking score of compounds for *S. aureus* (1Q1Y).

- **1Q1Y:** The entire 30 1, 3 Thiazine derivatives bind with 1Q1Y protein (crystal structure of peptide deformylase) and interaction was observed. Ptt3OCH3 showed the highest MolDock Score -96.683 as compared with reference drug Ampicillin.

Figure 3: Docking complex of 4AMV 5a.

Figure 4: Docking complex of 1Q1Y with 5d.

Scheme: Synthesis of 1, 3-thiazine derivatives

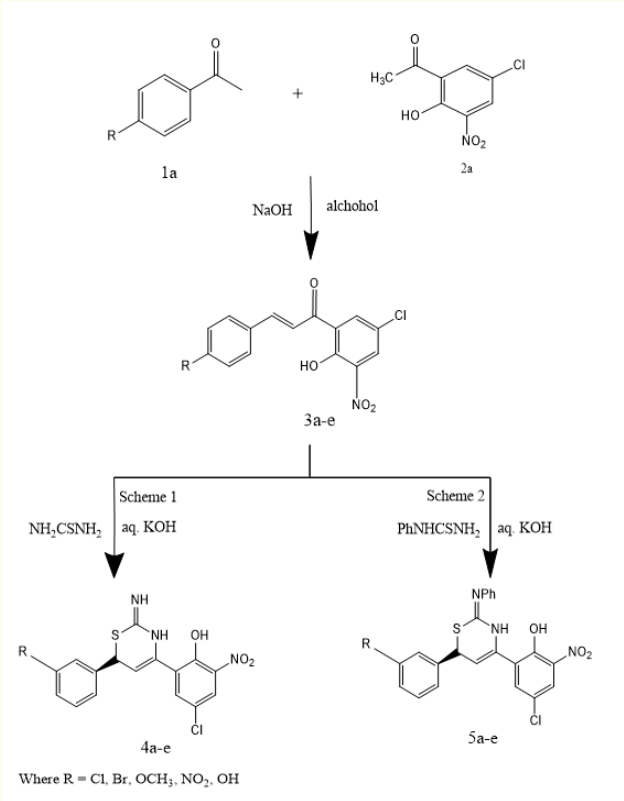


Figure d

Synthesis of 1, 3-thiazine derivatives

Steps for the synthesis of designed compounds

Preparation of 2-hydroxy-3-nitro-5-chloroacetophenone (2a)

2-Hydroxy-5-chloroacetophenone (3g) was dissolved in glacial acetic acid (3ml). Nitric acid was added to the mixture. The temperature was maintained below 0°C.Then stand for 1 hour. Then poured it into ice cold water and stirred it continuously. Then filtered, dried and crystallized from ethanol.

Preparation of 2-hydroxy-3-nitro- 5-chlorochalcones (3a-e)

2-Hydroxy-3-nitro-5-chloroacetophenone 2a (0.1M) was dissolved in ethanol (50 ml) and then derivatives of benzaldehyde (0.1M) were added to the reaction mixture and then heated up to boiling. Then drop wise NaOH (40%, 40 ml) was added and stirred it properly. Then reaction mixture was stirred mechanically for half an hour at room temperature and kept it for overnight. After that acidified it by HCl solution. Filtered the mixture and washed with Na_2CO_3 and water. The product was crystallized from ethanol and acetic acid mixture.

Preparation of 4-(2-hydroxy-3-nitro-5-chlorophenyl) -6 -(4-chlorophenyl) -2-imino -3, 6 dihydro-1, 3-thiazines (4a-e)

2-Hydroxy-3-nitro-5-chlorochalcone 3a-e and thiourea (0.01M) were dissolved in ethanol (25 ml) and KOH solution (0.02M) was added. Then mixture was refluxed for 2.5 hours diluted it with water and acidified with HCl. The product was filtered, and crystallized (4a-e).

Preparation of 4-(2-hydroxy-3-nitro-5-chlorophenyl) -6- (benzyle) -2-iminophenyl - 3, 6 dihydro-1, 3-thiazine (5a-e)

2-hydroxy-3-nitro-5-chlorochalcone 4a-e, (0.01M) was dissolved in ethanol (25 ml) and added to phenylthiourea (0.01M) then aq. KOH (0.02M) was added. Then mixture was refluxed for 2.5 hours diluted it with water and acidified with HCl. The product was filtered, dried and crystallized (5a-e).

The synthesized compounds (4a, 4b, 4c, 4d, 4e, 5a, 5b, 5c, 5d, 5e) were characterized on the basis of physicochemical properties and spectroscopy studies.

Determination of physicochemical properties

The various physicochemical properties of the synthesized compounds were determined:

- Melting point
- R_f value
- Solubility
- Partition Coefficient.

Melting point

The melting point of synthesized compounds was determined by open capillary method. Melting Points are reported in table.

Rf value

R_f value of synthesized compounds and their intermediate were determined by thin layer chromatography (TLC) using silica gel 60 F 254. Hexane: Ethyl acetate (9:1) was used as a solvent system. The spots were applied on a silica gel plate and the plate was run in a closed chamber. The spots on the plate were detected in iodine chamber and R_f value are reported in table.

Solubility

The Solubility was determined in various solvents and all compounds were soluble in Ethanol, water, ethanol, chloroform, benzene, and hexane qualitatively.

Partition coefficient

Partition Coefficient of synthesized compounds was determined by using octanol: water in equal ratio and Log P of compounds are reported in table.

Compound Code	R	Partition Coefficient (Log P)	M.P(^{0}C)	% yield	Rf value
Tt3Cl	Cl	0.311	120-122	76	0.55
Tt3Br	Br	0.170	145-147	68	0.58
Tt3OCH$_3$	OCH$_3$	0.314	102-106	58	0.68
Tt3NO$_2$	NO$_2$	0.277	138-140	70	0.64
Tt3OH	OH	0.120	132-135	51	0.72
Ptt3Cl	Cl	0.130	110-112	74	0.69
Ptt3Br	Br	0.243	157-160	75	0.49
Ptt3OCH$_3$	OCH$_3$	0.440	142-145	54	0.51
Ptt3NO$_2$	NO$_2$	0.510	138-140	60	0.35
Ptt3OH	OH	0.590	140-142	65	0.55

Table 4: Physicochemical properties of synthesized compounds:

Microbiological assay

Microbiological assay is a comparison of the inhibition of growth of microorganisms between the measured concentrations of the test compounds which is produced by known concentrations of a standard preparation of the antibiotic having a known activity. There are various methods which are used to determine the effectiveness of antimicrobials. There are various method of microbiological assay which are as follow.

- Diffusion method
- Paper disc diffusion method
- Cylinder or Cup-plate method
- Agar plate method
- Turbidity method
- Agar streak dilution method
- Serial dilution method.

The anti-bacterial activities of synthesized compounds were determine against one gram- positive bacteria (*S. aureus*) and one gram- negative bacteria (*E. coli*) by Agar disk diffusion method and the zone of inhibition was determined. In this test, wafers containing antibiotics was placed on an agar plate and bacteria were also placed on it and incubate it. The antibiotics stop the growth of the bacteria or kill the bacteria and the zone of inhibition was visible. Then zone of inhibition was measured.

Microbiological evaluation of synthesized compounds By Agar plate method

Standardization - Inoculation is made with a broth culture diluted to match a 0.5 McFarland turbidity standard, which is roughly equivalent to 150 million cells per ml.

Preparation of media: The media used in this method must be Mueller-Hinton agar at only 4 mm deep, poured into either 100 mm or 150 mm Petri dishes. The pH level of the agar must be between 7.2 and 7.4.

Incubation procedure

- A sterile swab was placed into the broth culture of organism and the excess liquid was removed and by using swab, the Muller- Hinton agar plate was streak to form a bacterial lawn.

S. N.	Ingredients	Nutrient broth
1.	Beef Extract	2.0 gm
2.	Casein hydolysate	17.5gm
3.	Starch	1.5gm
4.	Agar	17.0 gm
5.	Distilled Water	Up to 1000ml

Table 5: Composition of Mueller-Hinton agar.

pH of medium adjusted to neutral at room temperature.

Reference standard: Azethromycine (15µg/disc).

- Then the plate was allowed to dry for 5 min. Use an antibiotic disc dispenser to dispense discs containing specific antibiotics on to the plate and the plate was incubated overnight at 37°C.

Reading and interpreting results

The zone of inhibition was measured by first placed the plate on a white surface. Then with the help of ruler the zone of inhibition was measured from edge to edge crossing through the center and measure the diameter in mm.

Results and Discussion

The experimental work involved Docking studies, ADMET prediction, analysis of designed compound, elemental analysis, bioactivity score prediction, synthesis, characterization, and antimicrobial evaluationIn the present work totally 10 compounds were synthesized in two scheme. Step 1 involves the formation of chalcones from 2-Hydroxy-3-nitro-5-chloroacetophenone. The step 1 product (chalcone) reacted with thiourea or phenyl thiourea in presence of potassium hydroxide undergo cyclization to form 1, 3-thiazine derivatives. Molecular docking studies were carried out for the synthesized compounds against *E. coli* Glucosamine -6 P Synthase in Complex with Fructose -6 P (PDB ID- 4 AMV) and Crystal structure of Peptide deformylase from *Staphylococcus Aureus* Complex with Actinonin (PDB ID- 1Q1Y) by using Molegro Virtual Docker software which gives an insight in to the binding modes for the various inhibitors. Out of 10 inhibitors analyzed 5a has showed Mol Dock Score of -110.723 for *E. coli* and 5d has showed Mol Dock Score of -96.683 for *S. aureus*. The characterization of all ten synthesized compound were performed and structures were confirmed by ^{1}HNMR and Mass spectroscopy. Then all ten derivatives of 1, 3-thiazine were screened for their antimicrobial activity against gram positive bacteria viz. *S. aureus* and gram negative bacteria viz. *E. coli* species at conc. of 1000 µm azethromycine as a standard using agar plate techniques. The zone of inhibition formed were measured in mm and shown in table.

Docking score of synthesized compounds

Compound code	MolDock Score	Rerank Score	HBond
Tt4Cl	-86.7749	-67.5634	-6.83585
Tt4Br	-88.8064	-74.5592	-6.18152
Tt4OCH3	-93.706	-76.7804	-3.95941
Tt4NO2	-86.8956	-58.2635	-9.75651
Tt4OH	-106.654	-81.0678	-12.0967
Ptt4Cl	-110.723	-78.4613	-5.48575
Ptt4Br	-82.4321	19.6867	-6.4191
Ptt4OCH3	-86.7144	19.3956	-6.50447
Ptt4NO2	-88.6181	10.7845	-6.41836
Ptt4OH	-81.9273	19.5981	-6.47967

Table 6: Summary of docking score of synthesized 1,3-thiazine derivatives against target *E. coli* Glucosamine- 6 PSynthase in Complex with Fructose -6 P.

Compound code	Mol Dock Score	Rerank Score	HBond
Tt4Cl	-83.9601	-66.2623	-4.97171
Tt4Br	-84.5862	-66.6038	-4.37323
Tt4OCH3	-87.6422	-70.5666	-6.48139
Tt4NO2	-85.0538	-69.3519	-4.87202
Tt4OH	-83.252	-66.2025	-3.71184
Ptt4Cl	-87.4778	-66.6463	-7.1557
Ptt4Br	-92.1642	-75.5591	-4.89751
Ptt4OCH3	-95.4961	-77.3788	-3.69138
Ptt4NO2	-96.5073	-80.1216	-4.02885
Ptt4OH	-94.7954	-78.3154	-5.3336

Table 7: Summery of docking score of all synthesized 1, 3-thiazine derivatives against the target Crystal structure of Peptide deformylase from *Staphylococcus Aureus* Complex with Actinonin.

Microbiological evaluation of synthesized compounds

The antimicrobial evaluation of 1, 3 -thiazine derivatives conclude that the synthesized compounds were shown the potent

antimicrobial activity against *E. coli* and *S. aureus* respectively as compared to Azithromycin as a reference. And it also be concluded that 1, 3- thiazine derivatives posses more antimicrobial activity on gram negative *(E. coli)* bacteria than gram positive bacteria *(S. aureus).*

Compound Code	Zone of inhibition(mm)	
	E. coli	*S. aureus*
Tt4Cl	17	16
Tt4Br	20	17
Tt4OCH3	23	19
Tt4NO2	19	17
Tt4OH	25	15
Ptt4Cl	26	19
Ptt4Br	17	18
Ptt4OCH3	19	19
Ptt4NO2	20	21
Ptt4OH	17	21

Table 8: Antimicrobial activities of all synthesized 1, 3- thiazine derivatives.

The synthesized compounds were shown the potent antimicrobial activity against *E. coli* and *S. aureus* respectively as compared to Azithromycin (standard reading ≥ 18 mm) as a reference.

Conclusion

From last decades, the rise in drug resistant bacterial infection has become a very serious health care problem. Drug-resistance is generally acquired by the genetic mutation, this process takes long time but once the resistance is acquired, it spreads throughout the species. The infections once easy to treat are becoming difficult due to resistance development, and it became major problem for the researchers to design the molecules which can overcome resistance. Therefore, there is a need for the development of new agents which have better activity.

Thiazine derivatives are important and useful drugs. The thiazine derivatives are known to possess antibacterial, antifungal, antiviral, activities. Some benzimidazole containing marketed drugs are thiabendazole, norastemizole, telmisartan, omeprazole etc. The literature show that the thiazine derivatives substituted at position 2,3and 4 of phenyl ring attached at 6 position of thiazine ring show significant activity against bacteria, fungi etc. which encouraged the design and synthesis of some new substituted thiazine derivatives active against bacteria.

The 4-hydroxy-phenyl thiourea thiazine and 3-methoxy-phenyl thiourea thiazine derivatives were synthesized and purified by recrystallization. The layer chromatography was used for monitoring the reaction. Hexane: Ethyl (9:1) was used as a solvent system and spot were observed in Iodine chamber. Melting point was determined by open capillary method. The characterization was performed and structure of all compounds was confirmed by ^{1}H NMR and mass spectroscopy. The ^{1}HNMR spectra were recorded using DMSO-d6 as the solvent and TMS as internal standard. The Mass spectroscopy was recorded by using Bruker, micro-TOF-Q II 10348 and ESI-MS technique for ionization of samples.

The anti-microbial activity of product was evaluated against two different strains of micro-organisms; one gram-positive bacteria *(S. aureus),* and one gram-negative bacteria *(E. coli).* The antimicrobial evaluation was performed by agar plate method and zone of inhibition was measured in mm.

The result of microbiological evaluation revealed that ten different 1, 3 Thiazine derivatives were synthesized from chalcones and thiourea or phenyl thiourea. The products were checked for their anti-microbial activity by molecular docking studies against *E. coli* and *Staphylococcus Aureus* by using Molegro Virtual Docker software. Then *in-vitro* evaluation of anti-microbial activity of all compound of 1, 3 Thiazine derivatives were performed and all having considerable anti-microbial activity. From the above results it would be concluded that 1, 3-thiazines derivatives possess more anti-microbial activities on gram-negative *E. coli* bacteria than gram-positive bacteria *S. aureus*. The parent nucleus further could be explored to obtain more potent derivatives.

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