Design, Synthesis and Evaluation of Thiazolidines derivatives as Anti-bacterial Agents

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ABSTRACT

Through the use of QSAR modelling and docking analysis, this research investigates the structure-activity connection of new thiazolidine derivatives. For the purpose of developing QSAR models for antibacterial (Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Salmonella typhimurium) and antifungal (Candida albicans, Aspergillus species, and Cryptococcus neoformans) strains, the Fujita-Ban, Hansch-Mixed, and MOPAC techniques were used. The activity was greatly impacted by important characteristics as HOMO, LUMO, Sigma, and molecule structure. The in-vitro antimicrobial screening revealed that the compounds SP-25 (B. subtilis), SP-20 (S. aureus, E. coli), and SP-26 (S. typhi) had minimal inhibitory concentration (MIC) values that were superior to those of conventional medications. Compared to Greseofulvin, the antifungal activity of compound SP-10 was found to be equivalent. It was shown via docking experiments that efficient binding interactions with microbial enzymes (PDB: 2Y66 and 1IYL) were established, which provided support for the prediction accuracy of QSAR models. The realisation of these facts contributes to the rational creation of powerful thiazolidinone analogues that possess increased antibacterial capabilities.

Keywords: LUMO, MIC, NMR, HPLC, MOPAC & Anti-malarial activity.

1. Introduction

Antimicrobial drugs are the greatest contribution of the 20th century to therapeutics. Their advent changed the outlook of the physician about the power drugs can have on diseases. They are one of the few drugs which can cure, and not just palliate disease [1]. Their importance is magnified in the developing countries, where infective diseases predominate. As a class, they are one of the most frequently used as well as misused drugs [2].The emergence of bacterial resistance to existing antimicrobial agents represents a significant and growing

global health threat. Infections that were once treatable with common antibiotics are becoming increasingly difficult to manage, underscoring the urgent need for the discovery of new antimicrobial compounds. Given the complexity and dynamic nature of bacterial resistance, novel strategies are required for designing and developing effective antimicrobial agents[3].

One promising approach lies in the rational design of new molecules, which can be accelerated through computational techniques like molecular docking and Quantitative Structure-Activity Relationship (QSAR) modeling.Molecular docking, a technique that simulates the interaction between a small molecule (ligand) and a biological target (receptor), has revolutionized the drug discovery process [5]. By predicting how a drug might bind to its target site, docking studies offer valuable insights into binding affinity and the mode of action. This enables researchers to screen large compound libraries virtually and prioritize those most likely to have strong biological activity, thus saving time and resources.Among the many promising chemical scaffolds, thiazolidine derivatives have shown notable antibacterial properties, making them attractive candidates for further investigation. These compounds are versatile and can be modified to enhance their biological activity and pharmacokinetic properties. The design of novel thiazolidine derivatives with increased antimicrobial potency is a promising strategy, but it requires an efficient approach that integrates both experimental and computational methodologies [6].

In this research, we aim to design, synthesize, and evaluate thiazolidine derivatives as potential antibacterial agents, using a combination of molecular docking and QSAR modeling to guide the development of more effective compounds. The study will incorporate both in vitro and in silico approaches to identify and optimize thiazolidine-based molecules for antimicrobial activity [7].

The specific objectives of this study include Design novel thiazolidine derivatives with potential antimicrobial properties. Synthesize and characterize these derivatives using various spectroscopic techniques (NMR, IR, MS).Evaluate their antimicrobial activity through in vitro testing Perform molecular docking studies to explore the binding interactions between the thiazolidine derivatives and key bacterial targets, particularly those involved in bacterial cell wall synthesis [8]..Develop QSAR models to correlate the structural features of the thiazolidine derivatives with their antibacterial activity, providing a predictive tool for optimizing future compounds.By combining computational docking studies with QSAR modeling, this research aims to accelerate the development of new, potent thiazolidine-based antimicrobial agents [9]. The integration of these in silico methods with experimental antimicrobial testing provides a comprehensive framework for the rational design of more effective drugs in the fight against antibiotic-resistant infections.

2. Materials and Method

2.1 Synthesis of thiazolidinone derivatives

Two millimoles of 2-amino-5-chlorophenol M/s CDH,New Delhi, India one millimole of thioglycolic acid M/s Swaroop, Uttar Pradesh,India and one millimole of substituted aldehydes were combined in DMF M/s CDH,New Delhi, India with up to three drops of glacial acetic acid. The mixture was heated with stirring at reflux condition for 2-3 hours after being transferred to a 100 mL round-bottom flask. Thin layer

chromatography (TLC) using a 5% ethyl acetate:n-hexaneM/s CDH,New Delhi, Indiagradient allowed us to track the reaction's progress to completion as shown in **Figure 1**. The final product was transferred to crushed ice, filtered, and then extracted using a mixture of petroleum ether and ethyl acetate at a ratio of 2:8, with a volume of 3×10 ml. Vaccumwas used to condense the mixed solvent extracts. To get the pure product, the chemicals were recrystallised from ethanol.

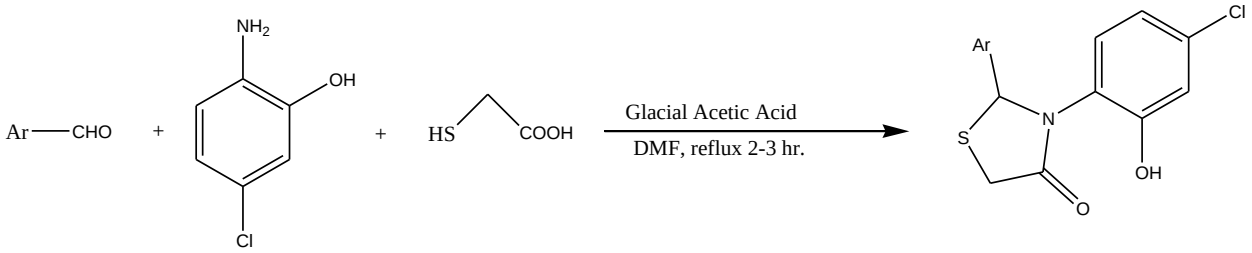


Figure 1: Schematic representation of thiazolidinone derivatives

2.2 Software and Tools

In order to create the three-dimensional models of the compounds, the two-dimensional models were created in Chemdraw Ultra 8.0 and then used in Chem3D Ultra. Molecular Mechanics 2 (MM2) and Molecular Orbital Packaging (MOPAC) were used to minimise energy using a Root Mean Square (RMS) variation value of 0.001. Then, 3D characteristics were computed using the same Chem3D Ultra Software produced by Cambridge Soft. Using the regression analysis tool VALSTAT, we create all of the statistically significant correlation models between the dependent and independent data sets. In addition, the molecular docking experiments were conducted using Molegro Virtual Docker6.0, created by CLC drug discovery Workbench, using 3D chemical structures and a targeted enzyme structure (responsible for biological activity) retrieved from the Protein Data Bank (PDB)

2.3 QSAR on Antibacterial Activity

The QSAR models andthere statistical data generated by this method is satisfactory and significant. Positive values of descriptors in the model show their positive contribution towards the biological activity. On that basis we can estimate the type of substitution suitable for designing of more potent chemical equivalents.

Bacillus Subtilis

pMIC = [4.272(± 0.153)]
+i2c [0.425(± 0.553)]
+i4h [0.550(± 0.553)]

Escherichia Coli

pMIC= [4.185(± 0.103)]
+i4h [0.512(± 0.372)]
+i4mth [0.637(± 0.371)]

Staphylococcus Aureus

pMIC = [4.401(± 0.169)]
+i24dm [0.422(± 0.610)]
+i4h [0.820(± 0.610)]

Salmonella Typhimurium

pMIC = [4.221(± 0.104)]
+i4m [0.380(± 0.375)]
+i4mth [0.476(± 0.375)]

Where, i2c indicates 2-chlorophenyl group, i4h indicates 4-hydroxybenzyl group, i4mth indicates 4-methylthiazol-5-yl group, i24dm indicates 2, 4-dimethoxyphenyl group and i4m indicates 4-methoxy group.

2.4Molecular Modeling Study employing Molecular Orbital Package (MOPAC):

The QSAR models and statics generated via this approach clearly correlate the properties of compound with their biological activity. Positive contribution of properties like: HOMO, LUMO, MTI (Molecular Topological Indices) and Cluster count gives a basic rational for designing and provide a estimation towards the types of structural modification required for further designing of more potent analogous as shown in**Figure 2.**

Bacillus Subtilis

pMIC = [7.989(± 4.718)]
+Ih [0.378(± 0.527)]
+Il [0.437(± 0.583)]

Staphylococcus Aureus

pMIC = [9.832(± 5.254)]
+Ih [0.561(± 0.587)]
+Il [0.522(± 0.649)]

Escherichia Coli

pMIC = [10.078(± 3.071)]
+Ih [0.749(± 0.328)]
+Icc [0.044(± 0.072)]

Salmonella Typhimurium

pMIC = [9.284(± 2.666)]
+Ih [0.593(± 0.299)]
+Imti [5.560(± 7.763)]

Where, Ih indicates HOMO (Highest Occupied Molecular Orbital), Il indicates LUMO (Lowest Occupied Molecular Orbital), Icc indicates Cluster Count and Imti indicates Molecular Topological Indices.

Table 1: Statistical Data for MOPAC Approach

MOPAC				
Parameters	***B. Subtilis***	***S. Aureus***	***E. Coli***	***S. Typhi.***
r	0.601	0.671	0.839	0.805
variance	0.06	0.075	0.023	0.019
Std. error	0.246	0.274	0.151	0.139
F value	3.111	4.504	13.091	10.132
ICAP	0.140	0.140	0.183	0.163

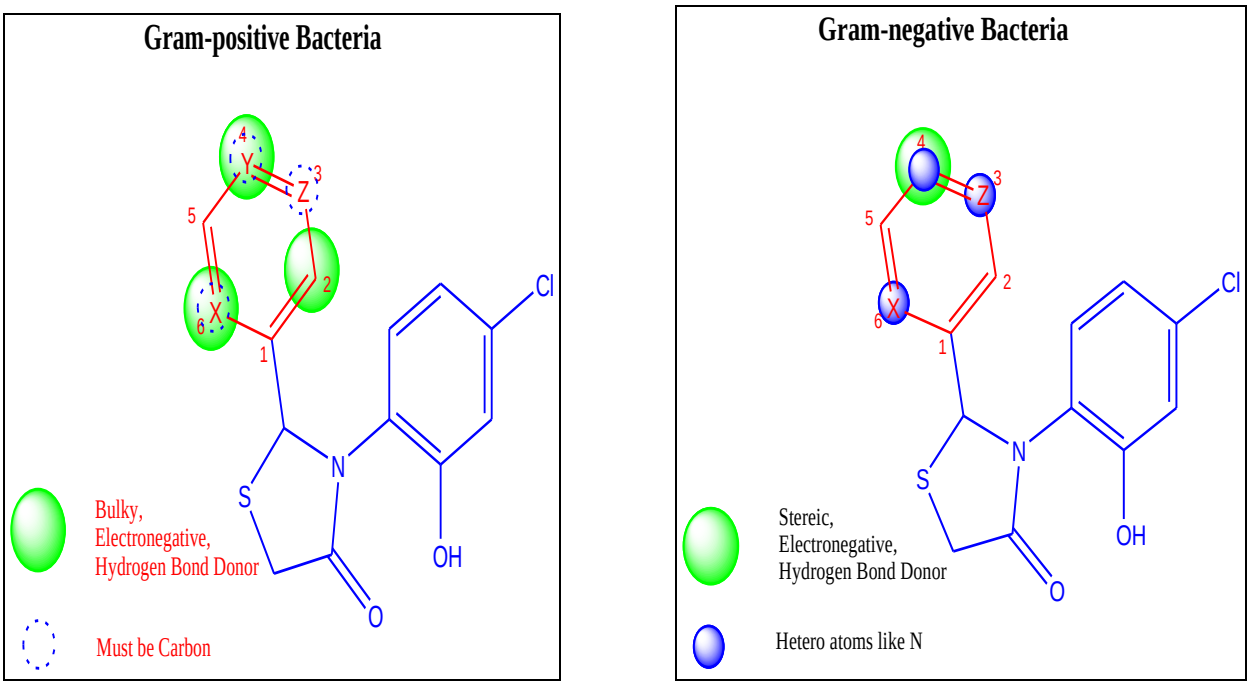


Figure 2: Proposed model for development of more potent antibacterial thiazolidinones

2.5 Antimicrobial Evaluation

At a temperature of 4 ºC, all of the bacterial strains are cultured in Mueller Hington Agar (MHA). The MIC measurements for the bacterial strains were carried out using a 96-well micro-titer plate reader using a serial macro-dilution approach. The bacterial colonies are incubated in Mueller Hington broth at 37 ºC on a rotary shaker at 160 rpm for 3-4 hours to generate a suspension that meets the McFarland standard 0.5 (OD at 625 nm is in between 0.08-0.13)

To conduct antibacterial testing, 96-well micro-titer plates are incubated at 37 ºC for 20 hours at 160 rpm with varying concentrations of compounds. The visual observation of inhibition of growth was used to identify the minimum inhibitory concentration (MIC) and record it. Potato Dextros Agar (PDA) is a medium that is often used to keep fungal strains alive. They calculated their MIC using the agar dilution method. Agar plates were used to directly distribute suspension cultures of each fungal strain with 0.5 McFarland standard. The doses of the investigated chemical ranged from 1.5 µg/mL to 150 µg/mL[11]. Within 48 to 72 hours, the plates are left

at room temperature to incubate. After incubation, the minimum inhibitory concentration (MIC) is determined by the absence of visible fungal growth on the agar plate. After inoculating with the studied compounds (dissolved in saline containing 5% DMSO) for 72 hours.

3 Result and Discussion

3.1 Designing of Compounds:

On the basis of reported structure activity relationship of Thiazolidinone analogues as antimicrobial agents, QSAR studies using Molecular Orbital Packaging (MOPAC) approaches, followed by docking studies on targeted enzyme structures, twenty seven compounds were screened and selected for synthesisas shown in **Figure 3 and Table 2.**

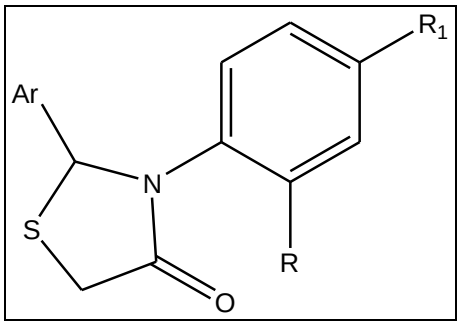


Figure 3: Common template for designing

Table 2: Designed Thiazolidinone analogues on the basis of computational studies

Compound No.	Ar	R	R1
SP-1	4- nitro Phenyl	H	Cl
SP-2	4-hydroxy Phenyl	H	Cl
SP-3	4-diethylamino Phenyl	H	Cl
SP-4	3,4,5-trimethoxy Phenyl	H	Cl
SP-5	4-ethyl Phenyl	H	Cl
SP-6	3,4-dimethoxy Phenyl	H	Cl
SP-7	3-ethoxy-4-hydroxy Phenyl	H	Cl
SP-8	3-nitro Phenyl	H	Cl
SP-9	3-hydroxy Phenyl	H	Cl
SP-10	4-dimethylamino Phenyl	H	Cl
SP-11	2,4-dimethoxy Phenyl	H	Cl
SP-12	2,5-dimethoxy Phenyl	H	Cl
SP-13	2-chloro Phenyl	H	Cl
SP-14	4-chloro Phenyl	H	Cl
SP-15	4-bromo Phenyl	H	Cl
SP-16	2-chloro-5-nitro Phenyl	H	Cl
SP-17	2-flouro-4-methoxy Phenyl	H	Cl
SP-18	3-chloro Phenyl	H	Cl
SP-19	2-chloro-5-nitro Phenyl	OH	Cl
SP-20	4-bromo Phenyl	OH	Cl
SP-21	4-dimethylamino Phenyl	OH	Cl
SP-22	4-diethylamino Phenyl	OH	Cl

SP-23	3-ethoxy-4-hydroxy Phenyl	OH	Cl
SP-24	3-hydroxy Phenyl	OH	Cl
SP-25	3-nitro Phenyl	OH	Cl
SP-26	4-diethylamino Phenyl	OH	OH
SP-27	4-diethylamino Phenyl	OH	H

3.2 Docking Analysis of designed compounds

Formation of cell membrane and wall of any species of microbe involves lots of enzymatic protein conversion and modification that can be employed for development of targeted and programed chemical inhibitors against microbes**As shown in Table3, 4.** [13]. The present work involves the similar approach especially against two types of enzymes N-myristoyltransferase (Nmt) and UDP-N-acetylmuramoylalanine—D-glutamate ligase (MurD ligase) which are vitally participates in the formation and functioning of cell membrane and cell wall of any fungal species (with special reference to *Aspergillus* species majorly responsible for fungal infections in humans and animal

Table3: Docking Scores of designed compounds on Pdb- 2Y66

Compound No.	MolDock Score	Rerank Score	HBond
SP-1	-97.3616	-77.5497	-4.36488
SP-2	-92.4535	-70.7773	-0.62065
SP-3	-104.263	-76.0327	-0.14039
SP-4	-98.401	-74.7662	-2.5
SP-5	-100.279	-73.0864	-
SP-6	-95.654	-72.5218	-0.24874
SP-7	-96.0426	-74.0293	-0.87852
SP-8	-103.433	-83.373	-4.02427
SP-9	-96.9923	-75.0362	-4.73801
SP-10	-96.4527	-75.369	1.442
SP-11	-97.3935	-70.8337	-2.66962
SP-12	-89.5545	-71.202	-
SP-13	-84.6198	-66.2008	-
SP-14	-92.8013	-70.8251	-0.59805
SP-15	-93.0274	-71.8186	-2.12828
SP-16	-97.7319	-75.1085	-1.25006
SP-17	-89.9247	-68.7421	-
SP-18	-93.6564	-71.3998	-1.03339
SP-19	-103.99	-75.2359	-7.0898
SP-20	-98.636	-75.4086	-4.99641
SP-21	-104.036	-73.6504	-4.93625
SP-22	-103.41	-76.8313	-3.06296
SP-23	-106.747	-78.2826	-5
SP-24	-99.1255	-76.4195	-4.98857
SP-25	-95.0463	-74.9942	-3.34681
SP-26	-102.858	-74.6683	-1.91242
SP-27	-100.777	-76.235	-3.86157

Table 4: Docking Scores of designed compounds on Pdb- 1IYL

Compound No.	MolDock Score	Rerank Score	HBond
SP-1	-115.323	-92.3488	-0.28331
SP-2	-117.565	-93.0857	-1.64637
SP-3	-130.296	-100.432	-2.096
SP-4	-123.647	-96.9	-2.07327
SP-5	-114.103	-88.4055	-
SP-6	-124.05	-99.8161	-
SP-7	-130.811	-103.486	-5.45223
SP-8	-126.612	-98.3335	-0.00238
SP-9	-122.922	-96.9135	-5.2449
SP-10	-117.66	-92.9734	-1.323
SP-11	-117.732	-95.0902	-
SP-12	-121.148	-96.1862	-
SP-13	-111.083	-86.036	-
SP-14	-112.574	-89.4774	-
SP-15	-107.66	-83.2677	-2.159
SP-16	-120.237	-99.7365	-2.35326
SP-17	-117.601	-92.2391	-
SP-18	-118.494	-89.8989	-
SP-19	-119.5	-90.3969	-6.68233
SP-20	-114.897	-88.7347	-2.1838
SP-21	-129.301	-97.5157	-2.34237
SP-22	-133.537	-103.555	-2.5
SP-23	-128.926	-98.6958	-5.6807
SP-24	-129.226	-104.083	-6.64904
SP-25	-125.756	-101.654	-4.22344
SP-26	-128.644	-102.265	-1.28484
SP-27	-140.621	-107.639	-6.19034

3.4 List of Compounds Selected for Synthesis

On the basis of structural modification suggested by Fujita, Hansch-mixed and MOPAC approaches and molecular docking studies, 10 most appropriate molecules were synthesized, characterized and evaluated for their antimicrobial activity**as shown in Figure 4 and 6.**

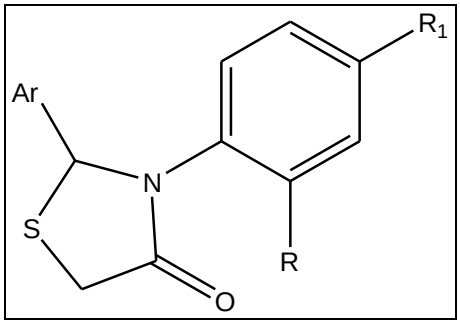


Figure 4: Common Template for synthesized compounds

Table 5: List of Synthesized Compounds

Compound No.	-Ar	R	R_1
SP-2	4-hydroxy Phenyl	H	Chloro
SP-3	4-diethylamino Phenyl	H	Chloro
SP-10	4-dimethylamino Phenyl	H	Chloro
SP-15	4-bromo Phenyl	H	Chloro
SP-20	4-bromo Phenyl	Hydroxy	Chloro
SP-21	4-dimethylamino Phenyl	Hydroxy	Chloro
SP-23	3-ethoxy-4-hydroxy Phenyl	Hydroxy	Chloro
SP-24	3-hydroxy Phenyl	Hydroxy	Chloro
SP-25	3-nitro Phenyl	Hydroxy	Chloro
SP-26	4-diethylamino Phenyl	Hydroxy	H

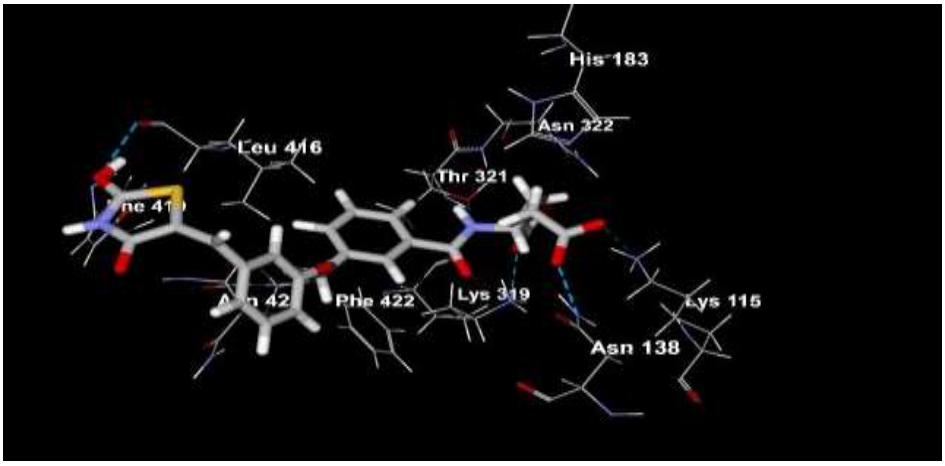
3.5 Docking Score of Synthesized compounds- (Pdb-2Y66)

The docking score of 10 synthesized compounds on the Pdb-2Y66 were given below. The compounds were shortlisted for synthesis, on the basis high Rerankscoresas show in **Figure 5 and Table 6.**

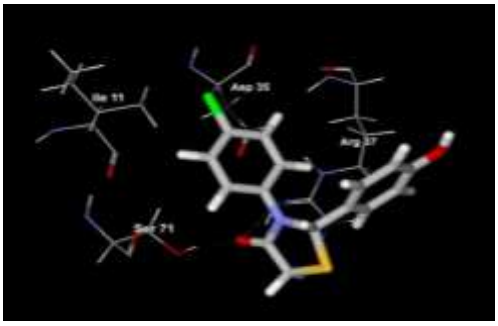
Table 6: Docking Score of synthesized compounds on Pdb-2Y66

Compound No.	MolDock Score	Rerank Score	HBond
SP-2	-92.4535	-70.7773	-0.62065
SP-3	-104.263	-76.0327	-0.14039
SP-10	-96.4527	-75.369	-1.442
SP-15	-93.0274	-71.8186	-2.12828
SP-20	-98.636	-75.4086	-4.99641
SP-21	-104.036	-73.6504	-4.93625
SP-23	-106.747	-78.2826	-5
SP-24	-99.1255	-76.4195	-4.98857
SP-25	-95.0463	-74.9942	-3.34681
SP-26	-102.858	-74.6683	-1.91242

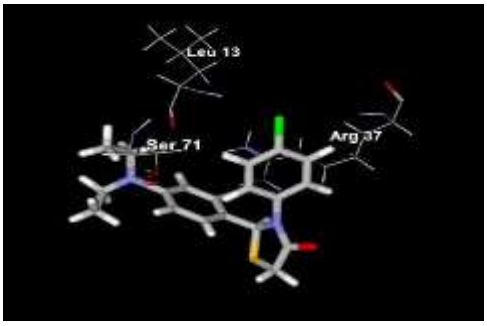
Docking poses



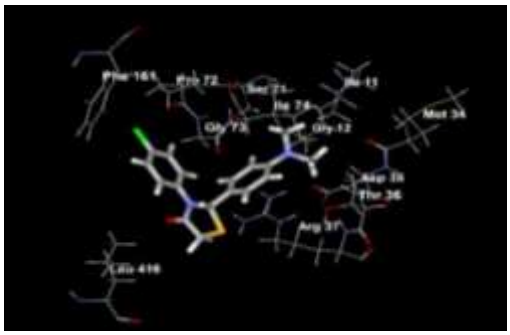
Reference Ligand



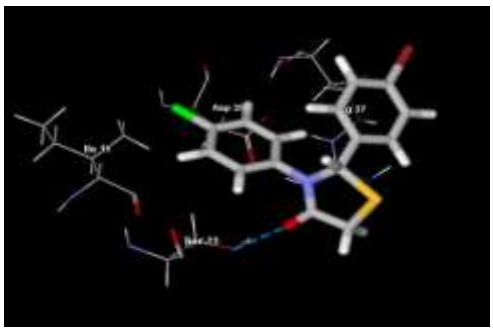
SP-2



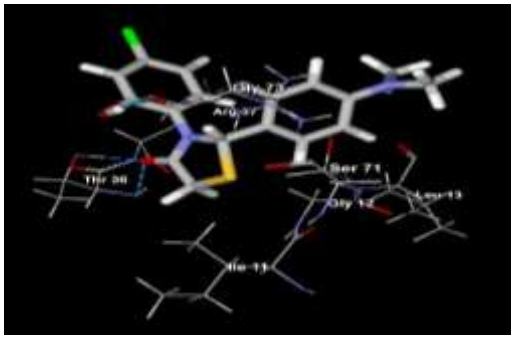
SP-3



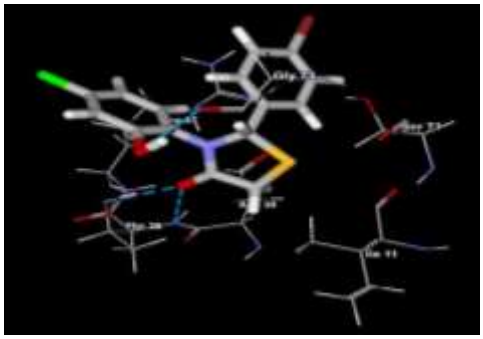
SP-10



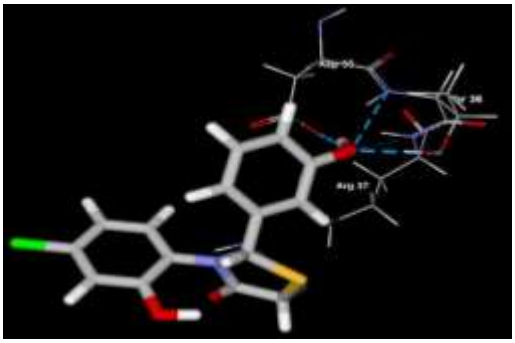
SP-15



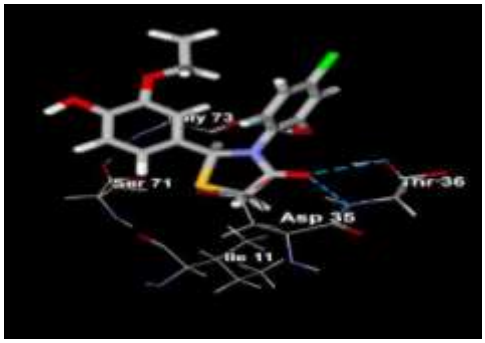
SP-20



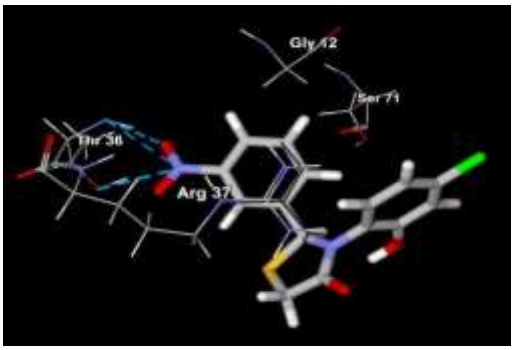
SP-21



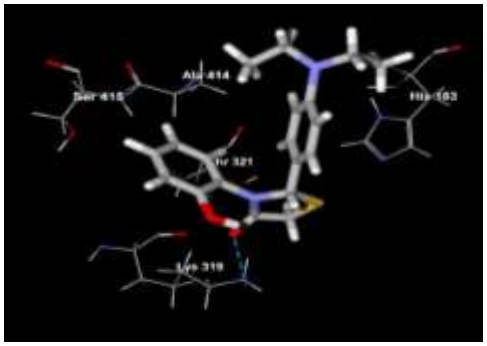
SP-23



SP-24



SP-25



SP-26

Figure5: Docking poses of the synthesised compound

3.6 Spectral Analysis of synthesized compounds

Synthesized compounds were then undergoing spectroscopic studies for the determination of their structures. Structures of all the synthesized Thiazolidinone derivatives have been established on the basis of their FT-IR, ^{1}H NMR and MASS Spectral data.

3.7 Fourier Transform- Infrared Spectroscopy (FT-IR)

Synthesized compounds were then studied under IR spectrometer for the detection of functional groups present in the structure. FT-IR spectrum bands of thiazolidinoneanalogues were already listed in Experimental section. Similar trend of stretching and bending vibrations are observed in all compounds as expected. Presence of C-Cl moiety in all compounds was confirmed by the peaks around 686.61 cm^{-1}region. Presence of C=O moiety in all compounds was confirmed by the peaks around 1700±50 cm^{-1}region. Presence of O-H moiety in all compounds was confirmed by the peaks around 3330 ±50 cm^{-1}region.Presence of Aromatic C (sp2) moiety in all compounds was confirmed by the peaks around 3131.81cm^{-1}region. Presence of $N(C_2H_5)_2$ moiety in Compound **SP-3** was confirmed by the peaks - Diethyl amino N-C stretch-3224.76 and C_2H_5:C-H stretch-1396.37; C-C stretch- 879.48, 1265.3; -CH_2- stretch- 1512.2 region. Presence of $N(CH_3)_2$ moiety in Compound **SP-10** and**SP-21** was confirmed by the peaks [14]: Dimethyl amino N-C stretch-3255.62; CH_3:C-H stretch-2885.84; C-H def.- 1357.79. Presence of C-Br moiety in Compound **SP-15** and **SP-20**was confirmed by the peaks:C-Br stretch- 516.89, 609.46. Presence of -OC_2H_5 moiety in Compound **SP-23** was confirmed by the

peaks: Ethoxy O-C stretch-1283.67; C_2H_5: C-H stretches- 1412.27; C-C stretch- 832.62, 1241.03;-CH2- stretch- 1512.2. Presence of N=O moiety in Compound **SP-25** was confirmed by the peaks: N=O stretch- 1542.66.

3.8 Mass Spectroscopy

Mass spectroscopy was performed on all ten synthesized compounds for the detection of mass. Results shows excellent outcomes which indicates that compounds were synthesized successfully. Molecular ion peaks M+, M+1 and M+2 peaks are found as shown in the table below.

Table 7: Mass Spectral Data of synthesized compounds

S. No.	Molecular Weight	MASS (m/z)
SP-2	305.78	306.231 (M+)
SP-3	360.9	360.27(M+)
SP-10	332.85	333.30 (M+1)
SP-15	368.68	370.18 (M+2)
SP-20	384.68	386.19 (M+2)
SP-21	348.85	349.32 (M+1)
SP-23	365.83	373.31
SP-24	321.78	322.23 (M+1)
SP-25	350.7	352.08 (M+2)
SP-26	342.46	344.2 (M+2)

3.8 In-vitro Anti-microbial Screening

3.8.1Activity against Gram Positive strains:

The synthesised compounds were tested for their antibacterial effects on two gram-positive pathogens,The findings were presented as MIC (µg/ml). Every one of the synthetic thiazolidinone derivatives worked, and a few of them worked even better than the gold standard medicine, ampicillin. While normal Ampicillin has an activity level of 250 ɛg/ml, this ranges from 62.5 to 500 µg/ml.

Table-8: Gram Positive Antibacterial inhibitory activity

S. No.	B. subtilis (µg/ml.)	S. aureus (µg/ml.)
SP-2	500	500
SP-3	100	100
SP-10	250	125
SP-15	500	200
SP-20	200	62.5
SP-21	100	125
SP-23	200	200

SP-24	200	250
SP-25	62.5	200
SP-26	125	200
Ampicillin	**250**	**250**

For ***Bacillus subtilis*** compounds **SP-3, SP-21, SP-23, SP-24, SP-25** and **SP-26** were found to be more active in comparison with the standard drug **Ampicillin** and compound **SP-2, SP-10** and **SP-15** were found moderate to equal active in comparison with **Ampicillin**. The result of in-vitro inhibitory activity and the suggestions of molecular modification by QSAR models were seen to be correlated. Negativity of Sigma (σ) indicates the requirement of electron donating groups and here in case of SP-3, SP-21, SP-23, SP-24 and SP-26 compounds there is a presence of strong electron donating groups.

For ***Staphylococcus Aureus*** compounds SP- 3, 10, 15, 20, 21, 23, 25 and 26 were found to have even better activity than standard drug **Ampicillin** and compounds SP-2 and SP-24 were found to have moderate to equal activity in comparison with **Ampicillin.** The result of in-vitro inhibitory activity and the suggestions of molecular modification by QSAR models were seen to be correlated. QSAR suggested the requirement of bulky and electron donating groups on para position and active compounds having the similar structural arena[15].

Activity against Gram negative strains:

The in-vitro compounds on two negative strains ***E. coli*** and ***S. typhi.***was carried out at Microcare laboratory Surat, Gujrat and the results were expressed in MIC (µg/ml) as shown in **Table 10**. The range of the activity is from 62.5 to 250 microgram/ml. and the activity of standard **Ampicillin** is 100 microgram/ml.

Table 9: Gram Negative Antibacterial inhibitory activity

S. No.	*E. coli* (µg/ml.)	*S.typhi.* (µg/ml.)
SP-2	125	100
SP-3	200	100
SP-10	250	250
SP-15	100	250
SP-20	62.5	100
SP-21	200	125
SP-23	250	100
SP-24	125	200
SP-25	200	100
SP-26	100	62.5
Ampicillin	**100**	**100**

For ***Escherichia coli*** compounds SP- 15, 20 and 26 were found to have equal or even better activity than standard drug **Ampicillin** and compounds SP-2, 3, 10, 21, 23, 24 and 25 were found to have moderate activity in comparison with **Ampicillin.** The result of in-vitro inhibitory activity and the suggestions of molecular modification by QSAR models were found to be correlated. QSAR suggested the negative contribution of Resonance effect (R) and in our most active compounds the substitution of Bromine (Br) on para position attracts the electron clouds from the phenyl ring and destabilized the resonance and finally increase the activity of compounds.

For ***Salmonella typhimurium***compounds SP- 2, 3, 20, 23, 25 and 26 were found to have equal or even better activity than standard drug **Ampicillin** and compounds SP-10, 15, 21 and SP-24 were found to have moderate activity in comparison with **Ampicillin.** The result of in-vitro inhibitory activity and the suggestions of molecular modification by QSAR models were found to be correlated. QSAR suggested the negative contribution of Sigma (σ) which favors to electron donating substitution and positive contribution of HOMO and Molecular Topological Indices (MTI) which favors soft nucliophile with higher topology. In our most active compounds the substitution of Methoxy and diethylamino on para position is bulky as well as electron donating in nature and finally increase the activity of compounds.

QSAR models were selected on the basis of highest value of correlation coefficient (r). Models generated on four bacterial strains ***Bacillus subtilis, Staphylococcus aureus, Escherichia coli*** and ***Salmonella typhimurium*** gives efficient and considerable Correlation coefficient (r) given

For ***Bacillus subtilis*** compounds **SP-3, SP21, SP-23, SP-24, SP-25** and **SP-26** were found to be more active in comparison with the standard drug **Ampicillin** and compound **SP-2, SP-10** and **SP-15** were found moderate to equal active in comparison with **Ampicillin**. The result of in-vitro inhibitory activity and the suggestions of molecular modification by QSAR models were seen to be correlated. Negativity of Sigma (σ) indicates the requirement of electron donating groups and here in case of SP-3, SP-21, SP-23, SP-24 and SP-26 compounds there is a presence of strong electron donating groups.

For ***Staphylococcus Aureus*** compounds SP- 3, 10, 15, 20, 21, 23, 25 and 26 were found to have even better activity than standard drug **Ampicillin** and compounds SP-2 and SP-24 were found to have moderate to equal activity in comparison with **Ampicillin.** The result of in-vitro inhibitory activity and the suggestions of molecular modification by QSAR models were seen to be correlated. QSAR suggested the requirement of bulky and electron donating groups on para position and active compounds having the similar structural arena.

3.8.3 Activity against Gram negative strains:

At the Microcare laboratory in Surat, Gujarat, the in-vitro antibacterial activity of the synthesised compounds was tested on two gram-negative organisms, E. coli and S. typhi. The results were presented as MIC (µg/ml). Every one of the synthetic thiazolidinone derivatives worked, and a few of them worked even better than the gold standard medicine, ampicillin. In comparison to the 100 microgram/ml activity of normal Ampicillin, the range of activity is 62.5 to 250 microgram/ml.

Table 10: Gram Negative Antibacterial inhibitory activity

S. No.	*E. coli* (µg/ml.)	*S.typhi.* (µg/ml.)
SP-2	125	100
SP-3	200	100
SP-10	250	250
SP-15	100	250
SP-20	62.5	100
SP-21	200	125
SP-23	250	100
SP-24	125	200
SP-25	200	100
SP-26	100	62.5
Ampicillin	**100**	**100**

For ***Escherichia coli*** compounds SP- 15, 20 and 26 were found to have equal or even better activity than standard drug **Ampicillin** and compounds SP-2, 3, 10, 21, 23, 24 and 25 were found to have moderate activity in comparison with **Ampicillin.** The result of in-vitro inhibitory activity and the suggestions of molecular modification by QSAR models were found to be correlated. QSAR suggested the negative contribution of Resonance effect (R) and in our most active compounds the substitution of Bromine (Br) on para position attracts the electron clouds from the phenyl ring and destabilized the resonance and finally increase the activity of compounds.

For ***Salmonella typhimurium***compounds SP- 2, 3, 20, 23, 25 and 26 were found to have equal or even better activity than standard drug **Ampicillin** and compounds SP-10, 15, 21 and SP-24 were found to have moderate activity in comparison with **Ampicillin.** The result of in-vitro inhibitory activity and the suggestions of molecular modification by QSAR models were found to be correlated. QSAR suggested the negative contribution of Sigma (σ) which favors to electron donating substitution and positive contribution of HOMO and Molecular Topological Indices (MTI) which favors soft nucliophile with higher topology. In our most active compounds the substitution of Methoxy and diethylamino on para position is bulky as well as electron donating in nature and finally increase the activity of compounds.

4.Conclusion

Physicochemical characterization concludes that the compounds were synthesized successfully and the results are favorable. Antimicrobial screening concludes that for *Bacillus subtilis* compound SP-25 shows the highest activity even better than the standard drug Ampicillin. For *Staphylococcus aureus* and *E. coli* compound SP-20

shows highest activity even better than the standard drug Ampicillin. For *S. typhi*.Compound SP-26 shows shows highest activity even better than the standard drug Ampicillin.

Acknowledgment

I wish to express my sincere thanks to Santosh kumarshukla , Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Sciences and Research forproviding theinfrastructure forconduction of practical of the presented work.

Conflict of Interest: There isno conflictof interest in this the text.

Author Contributions:

All authors have equal contribution in the preparation of manuscript and compilation.

Source of Support: Nil Funding:

The authors declared that this study has received no financial support.

Informed Consent Statement: Not applicable.

Data Availability Statement:The data presented in this study are available on request from the corresponding author.

Ethical approval: This study does not involve experiments on animals or human subject

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