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In Vitro and Insilico Analysis of Inhibition Efficiency of Synthetic Random Copolyester with Chalcones Moiety Against Human Pathogens

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Abstract:
The parent compound of chalcone derivatives six synthetic compounds were screened against six human pathogens Invitro and selected one compound i.e PTSH (C-5) against three pathogens Proteus mirabilis (MTCC 1429), Staphylcoccus auresus (MTCC 96) and Enterobacter cloacae (MTCC 7408) with Minimum Inhibitory Concentration are 10µg/mL and their drug-likeness score is 0.5 which is accepted as drug like activity and Insilico analysis of binding affinities of PTSH to the above microbial targets 3TO7 are -4.71 kcal/mol, 4E7G are -6.74 kcal/mol, and 1FWJ (mimicked model) are -4.82 kcal/mol. The inhibition constant of the synthetic PTSH drug-like compound to the pathogenic organisms are S.aureus K_i= 354.74µM, E.clovae K_i=11.42µM, P.mirabilis K_i=290.81µM concentration is required to block this organism activity. We sµggest our compound (PTSH) C-5 is known to inhibit a major human pathogen which is similar to standard antibiotics which is available in the market.

Keywords: Chalcones derivatives, Copolyester, Human pathogens, Autodocking.

1.INTRODUCTION:

Chalcones is a common valuable precursor available naturally example in all vegetables, soya, fruits and synthesized reported by many researchers and the compound possess multifunctional biological activity especially bactericidal activities [1]. Human infectious diseases, especially with Staphylococcus aureus, Enterobacter cloacae, and Proteus mirabilis, are a major concern. Aminohydrolase a multisubunit, nickel-dependent enzyme involved in the hydrolysis of substrate urea into carbonate and ammonia which are further eliminated by another mechanism in living organisms [2]. This aminohydrolase majorly present in a lower organism such as Proteus mirabilis [2]. This enzyme involved causing disease in humans includes peptic ulceration, hepatic encephalopathy, and pyelonephritis [3]. Urease inhibitors such as imidazoles [4], phosphorodiamidates [5] and hydroxamic acid derivatives 1-3 [6] were stopped using in-vivo due to their toxicity, instability and severe side effects. Fluoroquinolones are a synthetic compound used in the treatment of human Respiratory infections and urinary tract and Gastrointestinal tract [7]. A norfloxacin from parent compound can inhibit urease enzyme activity in proteus families such as mirabilis and vulgaris [8] and also with levofloxacin and ciprofloxacin P.mirabilis inhibited [9]. In general, the UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) plays an important role in its existing environment [10] and involved primarily in the biosynthesis of cell wall fatty acids and other macromolecules in the bacteria, inhibition of this enzyme by fosfomycin causes the bacterial life in the end [11-13]. The other natural derived inhibitors such as tulip sides [14,15] sesquiterpene lactone cnicin [16,17]. Another natural source for Mura inhibitor is terrific acid from a fungal origin namely the Aspergillus terreus which inhibits the bacterial cell wall synthesis in Escherichia coli and Enterobacter cloacae [18]. Also, the inhibitor phosphomycin to this Mura enzyme in E.cloacae have shown to irreversibly binds the amino acid cysteine of the polypeptides in the cell wall of this organism [11].

Many bacterial pathogens especially Staphylococcus aureus is a gram-negative organism which is methicillin-resistant and positive for the multidrug resistance [19]. Pyruvate kinase is a novel drug target in methicillin-resistant Staphylococcus aureus. Inhibitor from the natural marine product Bis-indole alkaloids to the MRSA Pyruvate kinase is IC_{50} more than 5 µg/mL and this inhibitor acted as antitumor, antiviral [20,21], cytotoxic [22-24], antimicrobial [20,25,26], antifungal [22] and anti-inflammatory effects [27].

Drug resistance is a common phenomenon in current trend by using thousands of synthetic drugs against many human bacterial pathogens. As noted in bacteria, harbor the acquired resistance which is a result of a mutation in chromosomal genes due to the mechanism of decrease in drug uptake, activation of efflux mechanism and acquisition of external DNA coding for resistance through horizontal gene transfer. In order to overcome the common problems caused by the chemical compounds used in treating diseases caused by many bacteria showed in other studies which lack the information that inhibitory activity on MurA in Enterobacter cloacae, *Escherichia coli*, pyruvate kinase in Staphylococcus aurreus and urease in Proteus mirabilis by Chalcones derivatives, therefore, we proposed Invitro and docking model to inhibits this organisms using our synthetic Chalcones derivatives which we synthesized six random copolyesters, was characterized and confirmed these compounds were non toxic and stable showed in our previous study [28] and need to analyse for the antibacterial activity in Invitro and Insilico analysis.

2. MATERIALS AND METHOD:

Synthesis of monomer diols

The monomer diols like HHEP, MHEP, HIMP are prepared from acid catalyzed Clasien Schmidt polycondensation method [47] and modified and already reported[29], (Table 1).

Table. 1. Synthetic chalcones derivatives codes with their copolyester codes

S.No	Sample Code	Copolyester Code
1.	C1	PITI
2.	C2	PIGH
3.	C3	PIOM
4.	C4	PSGH
5.	C5	PTSH
6.	C6	PTOI

Synthesis of copolyesters

The copolyesters PITI, PIGH, PIOM, PSGH, PTSH, PTOI are prepared by modification of certain functional groups in the parent compound and synthesized using methods from our previous study [29].

Preparation of the test micro-organisms

This method follows the standard procedures for testing antimicrobial agents. Standard cultures of bacteria from the Microbial Type Culture Collection and Gene Bank (MTCC), INDIA. A Gram-positive bacterium; *Staphylococcus aureus* MTCC 96, Staphylococcus aureus MTCC 737, Enterobacter cloacae MTCC 7408, Klebsiella pneumonia MTCC 109, Salmonella typhi MTCC 98, Vibrio cholerae MTCC 3905 and Proteus mirabilis MTTC 1429.

Preparation of culture media

The Luria Bertani (LB) media was prepared by weighing 14 grams mixed with one liter of distilled water, autoclaved at 121°C for 15 minutes. The medium was later dispensed into 100 mm sterile agar plates and left to set. The agar plates were incubated for 24 hours at 37°C to confirm their sterility. When no growth occurred after 24 hours, the plates were considered sterile.

Agar-Well Diffusion Assay

A concentration of 1 mg/mL of the synthetic drugs (6 compounds) was designed for agar well diffusion assay. Cultures of *S.aureus E.cloacae, K.pneumoniae S.typhi, V.cholerae P.mirabilis* were inoculated in TCBS, Mueller Hinton agar plates by spreading on the surface using a sterile L-rod. The sensitivity testing of the synthetic compounds was performed using the agar well diffusion method [31] and the wells of 6 mm diameter and 5 mm depth were made on the solid agar using a sterile glass borer. Add 20 µl of each synthetic drugs dissolved in ethanol of stock concentration 1 mg/mL, dispensed in labeled wells and 5 µg Gentamycin (broad-spectrum antibiotic) was used as a positive control Ethanol was used as negative control. After spreading incubated the plates for 24 -48 hours at 37 °C. Twenty four (24-48) hours later, the zones of inhibition were measured using a ruler and the results reported in (mm).

Minimum Inhibition Concentration (MIC) Evaluation

The MIC was done with synthetic 6 compounds (Customized) that showed antibacterial activity in the agar well diffusion assay against 6 human pathogens. The final concentration used for the activity was (5,10,50,100, 250,500 µg/mL), and the 6 compounds were dissolved in ethanol for the final activity. Test microbes were suspended in 5 mL of PDB incubated overnight, after which 0.02 mL was added to all the test tubes and preparation incubated at 37°C for 18 hours. After incubation, from each tube was subcultured on nutrient agar to see if bacteria growth was inhibited (Minimum Bactericidal Activity). The MIC was defined as the lowest concentration of an antimicrobial that inhibition of growth was determined by measuring the absorbance at 570 nm using an enzyme-linked immunosorbent assay (ELISA) reader [32].

Drug-likeness properties of chalcone derivatives

To determine drug-likeness such as structural characters which determines similarities to known drug molecules, notably the molecular weight, Log P, number of hydrogen donor or acceptor polar surface area and rotatable bonds are included as drug-like molecule as documented [33].

ACD/ChemSketch for chemical structure-property analysis and Open Babel Converter

ACD/ChemSketch Freeware allows drawing chemical structures for this study compound 1 and 5 (PITI, PTSH) were selected based on above table criteria. It also includes calculation of molecular properties, density, molar refractivity 2D and 3D structures cleaning and viewing. Open Babel is used to facilitating the interconversion of chemical data from one format to another which is compatible to dock with proteins using respective docking software and the website for this program as http:// openbabel.org /docs/ current/ Introduction/ goals.htmL.

Selection of protein and preparation of its structure

Staphylococcus aureus MTCC 96 (PDB ID:3T07), Enterobacter cloacae MTCC 7408 (PDB ID:4E7G) The UreC protein (P17086) from Proteus mirabilis was not available in PDB database. Hence the sequence was modeled using SWISSMODEL-automated mode. The template structure urease (1FWJ) from *Klebsiella aerogenes* was superimposed with UreC modeled structure from *Proteus mirabilis* using SwissModel. The proteins were prepared for docking by the removal of water molecules.

Selection of ligand and preparation of its structure

We synthesized 6 compounds based our customized need and selected in this study. The structures of the ligands were docked with human pathogens active components. Hydrogen bonds were added and the energy was minimized using Autodock tool.

Interaction studies of binding

The exact fit of the ligand to a receptor was studied using AutoDock [34] and in the Discovery Studio Accelrys software [35]. The interactions of the compounds with the target were analyzed using the receptor-ligand interaction protocol. The receptor cavities were explored and the active site residues selected were used for the interaction with the 6 compounds and these interactions were viewed by Pymol software [36].

Data Analysis

The MIC for each microorganism was analyzed using one-way analysis of variance (ANOVA). P value < 0.05 was considered as significant. Student T-test also applied for the significance.

3. RESULTS AND DISCUSSION:

Antibacterial activity of synthetic compounds

Based on our need we modified the functional groups of each compound and synthesized by wet laboratory method. We also analyzed the solubility of the compounds which is main criteria for the compound to act as drug-likeness. Totally we synthesized 6 synthetic compounds and lyophilized those compound in final powdered form. The antibacterial activity of 6 synthetic compounds showed bacteriocidal activity against 6 pathogens such as Proteus mirabilis, Staphylococcus aureus, Enterobacter cloacae, Klebsiella pneumonia, Salmonella typhi and Vibrio cholera. Among these organisms and compounds, only compound 1 and 5 (PITI, PTSH) showed broad-spectrum activity against all pathogens but less in compound-5(PTSH) alone. Whereas other synthetic compounds showed very mild activity against this organism which is a very traceable level of inhibition (Table 2).

Table.2. Overall view of antibacterial activity screen for 6 synthetic compounds against 6 human pathogens

	Strain	C1	C2	C3	C4	C5	C6
1	*Klebseilla pneumoniae* MTCC 109	+	ND	ND	ND	ND	ND
2	*Salmonella typhi* MTCC 98	+	ND	ND	ND	ND	ND
3	*Vibrio cholerae* MTCC 3905	ND	ND	ND	ND	ND	ND
4	*Proteus mirabilis* MTTC 1429	+	ND	ND	+	+	ND
5	*Staphylococcus aureus* MTCC 96	+	ND	+	+	+	+
6	*Enterobacter cloacae* MTCC 7408	+	ND	ND	ND	+	ND

Table.3. The antibacterial activity of two compounds was selected based on the compound-specific activity against three human pathogens (Green color box).

	Strain	**C1**	**C5**
1	***Klebseilla pneumoniae* MTCC 109**	**+**	**ND**
2	***Salmonella typhi* MTCC 98**	**+**	**ND**
3	***Vibrio cholerae* MTCC 3905**	**ND**	**ND**
4	***Proteus mirabilis* MTTC 1429**	**+**	**+**
5	***Staphylococcus aureus* MTCC 96**	**+**	**+**
6	***Enterobacter cloacae* MTCC 7408**	**+**	**+**

Red Box- Broad spectrum of activity
GreenBox-Compounds specific activity

Table.4. The selected PTSH and PITI compounds for MIC in µg/mL were calculated compared to reference standards against 6 human pathogens.

	Strain	Zone of Inhibition	(MIC), µg/mL (C-1)PITI	Zone of Inhibition	(MIC), µg/mL (C-5) PTSH
1	*Klebseilla pneumoniae*	11 mm	50 µg/mL	ND	50 µg/mL
2	*Salmonella typhi*	10 mm	50 µg/mL	ND	50 µg/mL
3	*Vibrio cholerae*	ND	50 µg/mL	ND	50 µg/mL
4	*Proteus mirabilis*	10 mm	50 µg/mL	11 mm	10 µg/mL
5	*Staphylococcus aureus*	11 mm	50 µg/mL	12 mm	10 µg/mL
6	*Enterobacter cloacae*	11 mm	50 µg/mL	14 mm	10 µg/mL

Based on the 2 criteria 1) broad-spectrum activity 2) comparison of two compounds efficiency against selected 3 pathogens, we selected compound 1 and 5(PITI, PTSH) for further action to be checked (Table 3). The next step is the quantitation of compound used for the antibacterial activity against 3 specific pathogens result in compound 1(PITI) showed an 11-12mm well diffusion (killing zone) at the concentration of 50µg/mL whereas using compound 5(PTSH) the bactericidal activity was noted at the concentration of 10µg/mL (Table 4). This result showed that compound 5 is more specifically targeting the 3 human pathogens compared to compound 1(PITI). Therefore, we selected the synthetic compound 5 (PTSH) which is best for docking against these 3 pathogens virulence activity.

Invitro antibacterial activity using compound- 5 (PTSH)

An antibacterial activity represents agar well diffusion assay and zones of inhibition produced by the synthetic compounds depend on the individual compound functional group in it. This difference in inhibition varies between compounds targeted to different species [29]. The overall antibacterial activity in our study showed killing activity with compound-1 against Klebseilla pneumonia Salmonella typhi, Proteus mirabilis, Staphylococcus aureus, Enterobacter cloacae with 50µg/mL concentration and zone of inhibition is 10-11mm compound-2(PIGH) not showed any activity against 6 pathogens, C-3 (PIOM) has activity against Staphylococcus aureus at 50µg/mL with zone of clearance 14mm, C-4 (PSGH) showed Proteus mirabilis, Staphylococcus aureus at 50µg/mL concentration with clearance 10mm as shown (Table 4) but interestingly the C-5 (PTSH) showed activity against Proteus mirabilis ,Staphylococcus aureus, Enterobacter cloacae at 10µg/mL final concentration with zone of inhibition is 11-14 mm which is notable for further experiment to prove the

efficiency of inhibition and C-6(PTOI) compound showed activity against Staphylococcus aureus only. Among all these compound, we have selected one compound PTSH against 3 pathogens for the best antibacterial activity as shown in agar plates (Figure 1).

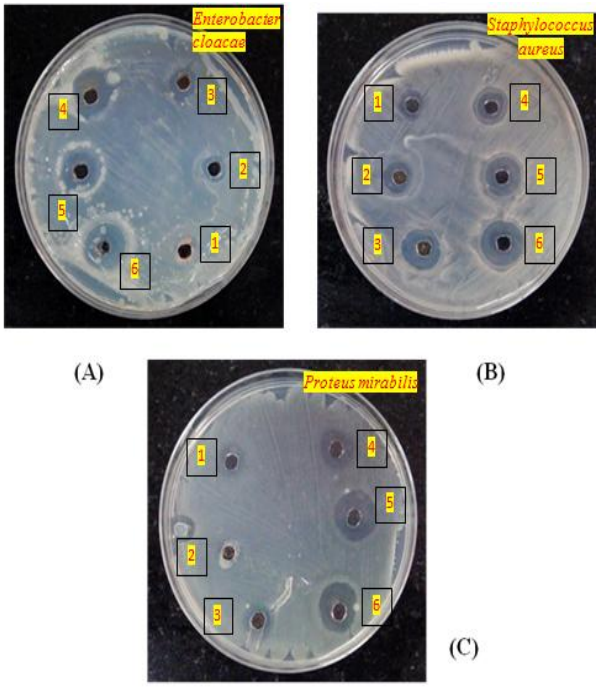


Figure.1. Zone of inhibition of C-5 (PTSH) test compound at 10 µg/mL against *Enterobacter cloacae* MTCC 7408. All tests were carried out in triplicates(A), the C-5 (PTSH) compound at 10 µg/mL against *Staphylococcus aureus* MTCC 96, (B) C-5 (PTSH) test compound at 10 µg/mL against *Proteus mirabilis* MTTC 1429(C).

Synthetic compound- 5 (PTSH) structural properties

The complex molecule of benzene mixture in the above structure (Table 2-4) of compound 5 (PTSH) which is selected by based on the broad spectrum activity and sensitivity against a 3 specific human pathogens showed a moderate to high inhibition with the final concentration of 10µg/mL. Any compound that is synthesized in a laboratory and yield from natural sources must exhibit two criteria 1) must not cause side effects 2) must be the least concentration which could inhibit the pathogens and must be stably inhibited. Therefore we used online tools to analyzed the synthetic compound synthesized is acted as a drug and specific for broad-spectrum or narrow spectrum inhibition (Figure 2).

Figure.2. Chemical structure of PTSH. (A) 2D picture of synthetic compound C-5(PTSH) with functional groups (B) 3D picture of C-5(PTSH) compound with major carbon backbone.

Drug-likeness of synthetic compound PTSH

The drug-likeness software like molsoft and highlighted on the website http://molsoft.com/mprop/ and other software swiss ADME http://www.swissadme.ch. [37] Proves the compound exhibits the drug in nature. First, in molsoft, the compound 5 after submission showed a graphical view (Figure 3) indicates three lines represents (Green) non-drugs, (Blue) standard drug and the red line indicates our compound 5. From this graph, we draw the conclusion that our compound 5 falls within the range of standard drugs are in blue line with the numerical value of 0.77 states C-5 (PTSH) behave like drug-likeness which is a promising result we obtained. The criteria are larger the drug-likeness score value is, the higher the chance of particular molecule will be very active. The LogP (octanol/water partition coefficient) must be Rmse=0.56 and LogS (water solubility) must be Rmse=0.87. Our compound falls beyond these values so C-5 (PTSH) is not water soluble compound. Second, using SwissADME our compound 5(PTSH) nature is analyzed and it must follow the criteria for a compound to act a good drug-likeness. In bioavailability, radar showed the drug-likeness and consists of 6 physiochemical characters such as lipophilicity, size, polarity, solubility, flexibility, and saturation. The pink area in which the radar plot of the molecule has to fall entirely to be considered drug-like.

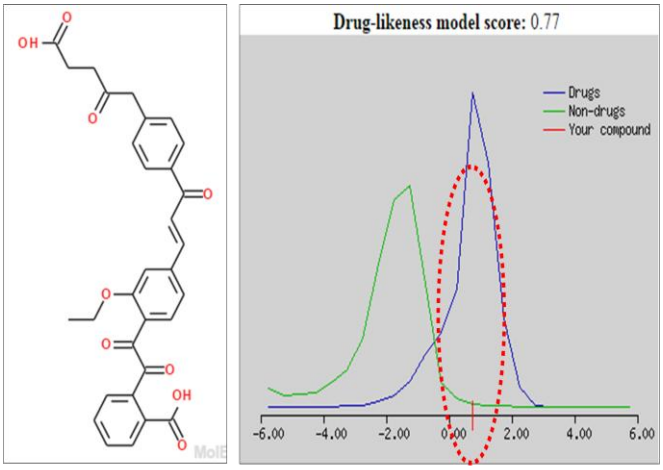
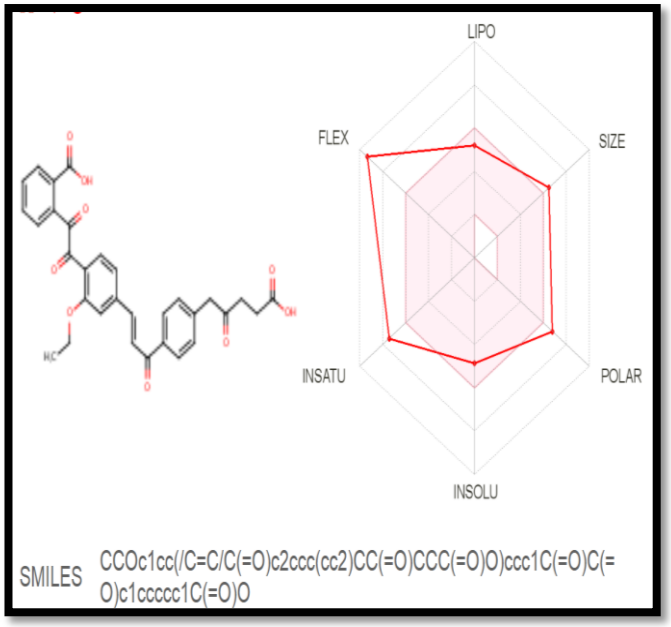


Figure 3. Synthetic compound 5 (PTSH) with drug-likeness analyzed using molsoft with the output of structure and properties

The individual parameters in radar describe that in Saturation, the ratio of sp3 hybridized carbon with total carbon is 0.25 and a molecular weight between 150 to 500 g/mol and polarity 20-130A^{o} and solubility log S not more than 6 and lipophilicity range from-0.7 to +6.0 and flexibility must be below 9 rotatable bonds. Any deviation in line from the pink area is considered as least drug-likeness.

Our compound 5 (PTSH) possess the lipophilicity with correct molecular weight and polarity is intact and solubility is under considerable range but the saturation and flexibility are out of focus to indicate totally minimal consideration that compound 5 (PTSH) acts less drug-likeness but exhibits inhibition activity against bacterial pathogens **(**Figure 1).



Physicochemical Properties	
Formula	C31H26O9
Molecular weight	542.53 g/mol
Num. heavy atoms	40
Num. arom. heavy atoms	18
Fraction Csp3	0.16
Num. rotatable bonds	14
Num. H-bond acceptors	9
Num. H-bond donors	2
Molar Refractivity	145.78
TPSA	152.11 Å²

Water Solubility	
Log S (ESOL)	-4.87
Solubility	7.24e-03 mg/ml ; 1.34e-05 mol/l
Class	Moderately soluble
Log S (Ali)	-6.47
Solubility	1.83e-04 mg/ml ; 3.38e-07 mol/l
Class	Poorly soluble
Log S (SILICOS-IT)	-7.58
Solubility	1.42e-05 mg/ml ; 2.62e-08 mol/l
Class	Poorly soluble

Druglikeness	
Lipinski	Yes; 1 violation: MW>500
Ghose	No; 2 violations: MW>480, MR>130
Veber	No; 2 violations: Rotors>10, TPSA>140
Egan	No; 1 violation: TPSA>131.6
Muegge	No; 1 violation: TPSA>150
Bioavailability Score	0.11

Pharmacokinetics	
GI absorption	Low
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-7.06 cm/s

Lipophilicity	
Log $P_{o/w}$ (iLOGP)	2.76
Log $P_{o/w}$ (XLOGP3)	3.59
Log $P_{o/w}$ (WLOGP)	4.61
Log $P_{o/w}$ (MLOGP)	1.81
Log $P_{o/w}$ (SILICOS-IT)	6.08
Consensus Log $P_{o/w}$	3.77

Medicinal Chemistry	
PAINS	1 alert: imine_one_A
Brenk	2 alerts: diketo_group, michael_acceptor_1
Leadlikeness	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5
Synthetic accessibility	4.08

Figure. 4. Computed parameter values are grouped in the different sections of the one-panel molecule output (PhysicochemicalProperties,Lipophilicity,Pharmacokinetics, Drug-likeness and Medicinal Chemistry) in Swiss ADME submission site for a synthetic compound 5(PTSH)**.**

Insilico analysis of the interaction of compound 5 with three pathogens active site

In-vitro analysis of antibacterial activity was shown that among 6 compounds after antibacterial screen it is noted that only 2 compounds were selected compound 1 and compound 5 (PITI, PTSH). Based on the selective concentration of compounds c-5(PTSH) is in the first line of antibacterial activity, since, it killing activity as low as at 10µg/mL is the optimized concentration at which the 3 pathogens were killed shown (Figure 1). This criterion is selected for studying the Insilico analysis of these 3 pathogens docked with compound 5(PTSH)(Figure 5). Briefly, we have selected the 3 pathogens from the PDB with ID and modeled the compound using chemsketch and the compound-5 (PTSH) were first converted into smiles file format and pasted and docked with 3 pathogens for their binding affinity with energy using Autodock tool. After docking the result of interaction were viewed through PyMol online software.

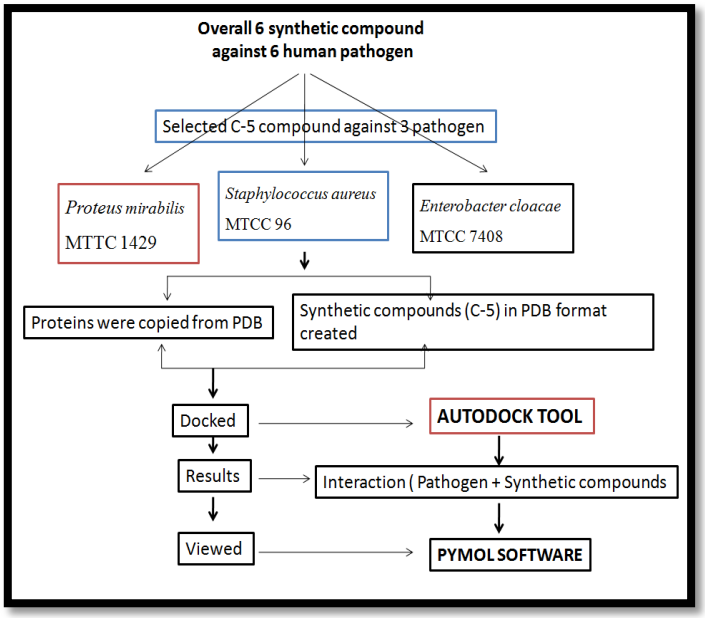


Figure.5. Schematic flow chart for the selection of compound and organism for Insilico analysis.

Staphylococcus aureus (PDB ID:3T07) is a multidrug resistance gram-negative bacteria is a common problem in clinical side shown (Figure 6). The pathophysiology of this bacterial colonization, the infection starts with cardiac endothelium, inflammation. This pathogen has the tendency to attach to extracellular matrix proteins, platelets, and fibrin [38]. Also, this organism induces platelet aggregation and activation [39]. These pathogens also noted that causes direct toxicity to the endothelial cells [40]. Due to this pathogen causes other diseases as Necrotizing fasciitis [41], Pyomyositis [42].

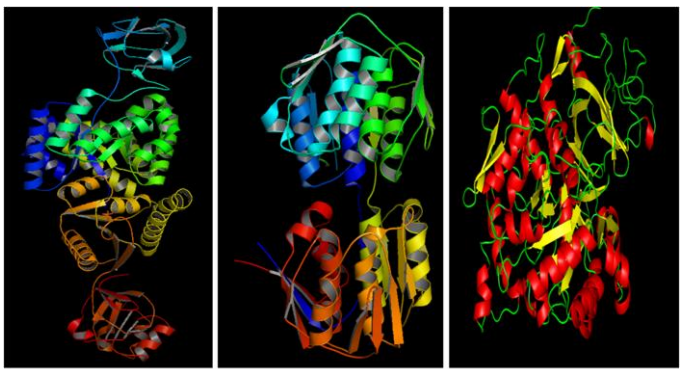


Figure.6. Crystal structure of three human pathogens S.aureus (Pyruvate kinase) PDB ID: 3T05, E.cloacae (MurA) PDB ID: 4E7G and P.Mirabilis (Urec) Modelled with 1FWJ which is reacted to compound 5 were viewed using PyMol software.

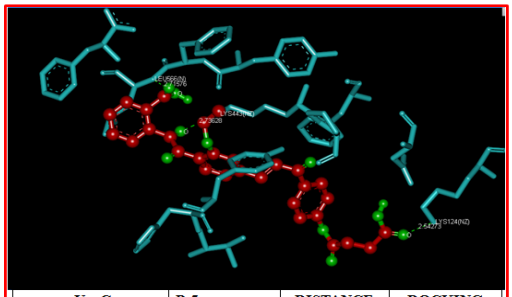
Enterobacter cloacae (PDB ID: 4E7G) is a facultative gram-negative bacteria grows in anaerobic condition family of Enterobacteriaceae. This pathogen has other types includes Enterobacter asburiae, Enterobacter cloacae, Enterobacter hormaechei, Enterobacter kobei, Enterobacter ludwigii, and Enterobacter nimipressuralis. Enterobacteria species is a nosocomial pathogen [43,44]. Enterobacter also an opportunistic bacteria causes infections in hospitalized

environments [45, 46]. E. cloacae are resistance to ampicillin, cephalosporins with showing mutation to resistance to these antibiotics [47, 48]. Proteus mirabilis a negative bacteria rod in shape which gives positive to urease, indole negative and lactose negative and has characteristic hydrogen sulfide production [49]. This organism causes urinary tract infections such as pyelonephritis and cystitis with type 2 diabetes patients [50,51]. In addition, this pathogen also causes urinary stones (urolithiasis), respiratory tract, eye, ear, nose, skin, throat, burns, and wounds and has been noted in neonatal meningoencephalitis, empyema, and osteomyelitis [49, 52], P. mirabilis using flagella to invade cells of urinary tract, including Vero [53].

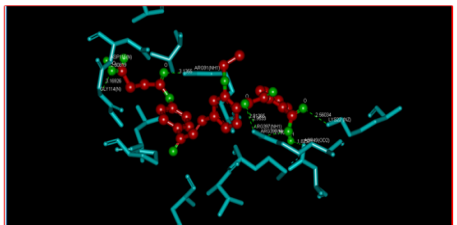
Docking of 3 human pathogens with Compound-5 (PTSH)

Insilico analysis of 3 human pathogens showed (Figure 7) is a good interaction to the active site of each pathogenic organism. In Proteus mirabilis, the UreC amino acid residues interact with compound-5 (PTSH) are lysine [124,443] and leucine [566] with the oxygen atom of C-5 (PTSH) with the docking energy of -4.82 (Kcal/mol) indicates very close interaction with each other. This showed C-5 (PTSH) act as a good antibacterial agent present as synthetic compounds similar to other plant sources. In Enterobacter species, the MurA protein active site amino acids residues interact with compound 5 as lys[22],Asp[49],Arg[397],Arg[91],Gly[114] and Asp[115] with C-5(PTSH) oxygen atom except for Asp49 interacts with the hydrogen atom of C-5(PTSH) with docking energy as -6.74 confirms the close interaction with this pathogen. Pyruvate kinase of Staphylococcus aureus amino acid residue interacts with C-5(PTSH) compound as lys[59], Ile[60,] and Arg[443] with oxygen atom in C-5 (PTSH) compound with docking energy of -4.71.

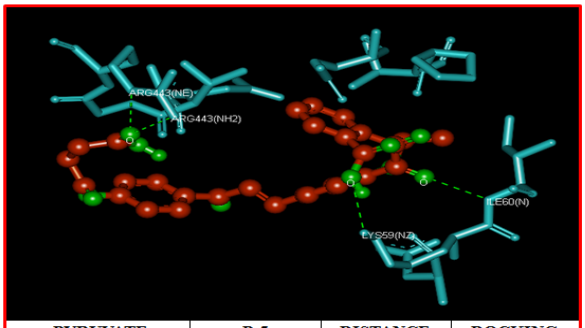
All these results confirm the specificity of synthetic compound-5(PTSH) towards these 3 pathogens interacts and kills these organism based on dose responses with the time need to be also considered.



UreC Proteus mirabilis		P-5	DISTANCE (Å)	DOCKING ENERGY (KCAL/MOL)
RESIDUE	ATOM	ATOM		
LYS124	NZ	O	2.54	−4.82
LYS443	NZ	O	2.73	
LEU566	N	O	2.71	



MURA ENTEROBACTER CLOACAE		P-5	DISTANCE (Å)	DOCKING ENERGY (KCAL/MOL)
RESIDUE	ATOM	ATOM		
LYS22	NZ	O	2.56	−6.74
ASP49	OD2	H	1.82	
ARG397	NH1	O	2.91	
ARG397	NH2	O	2.79	
ARG397	NH2	O	2.95	
ARG91	NH1	O	3.13	
GLY114	N	O	3.16	
ASP115	N	O	2.80	



PYRUVATE KINASE STAPHYLOCOCCUS AUREUS		P-5	DISTANCE (Å)	DOCKING ENERGY (KCAL/MOL)
RESIDUE	ATOM	ATOM		
LYS59	NZ	O	2.93	−4.71
ILE60	N	O	2.84	
ARG443	NE	O	2.73	
ARG443	NH2	O	3.18	

Figure.7. Three human pathogens were docked with compound-5 (PTSH) using Autodock tool with the result displayed docking energy and amino acids.

Table.5. Three organisms with inhibition constant after docking with the compound-5 (PTSH).

Pathogen	Compound	Ligand efficiency	Inhibition constant (µM)	Hydrogen bonds
S.aureus	C-5 (PTSH)	0.12	354.74	3
E.clovae	C-5 (PTSH)	0.17	11.42	4
P.mirabilis	C-5 (PTSH)	0.12	290.81	3

Comparison of Invitro concentration of compound 5 (PTSH) with Insilco analysis showed the matching amount of C-5 (PTSH) in micromolar indicates a good inhibition constant was fixed to inhibits these 3 pathogens. Especially S.aureus showed higher inhibition constant at 354.74 µM compared to P. mirabilis at 290.81 µM and both of this pathogen compared to E.clovae which is inhibited at an 11.42µM concentration only as shown (Table 5). This result indicates each compound especially, compound-5 has more species-specific inhibition of S.aures.

4. CONCLUSION

In the study, we have focused on six synthetic compounds, specifically, compounds 1, 5, (PITI, PTSH) that have significant inhibitory activities against three human pathogens Proteus *mirabilis*, Staphylococcus *aureus* and Enterobacter *cloacae*. we have shown four of other compounds are also moderately active against Staphylococcus *aureus* but have least activities against *Klebsiella pneumonia*, *Salmonella typhi*, *and Vibrio cholera* by agar well diffusion method. In MIC method, suggest that Compound 5 (PTSH) and compound 1(PITI) have higher antimicrobial activities (MICs, 10µg/mL, and 50µg/mL respectively) than the other synthetic compounds 2,3,4 and 6 (PIGH, PIOM, PSGH, PTOI) (MICs, more than 50 µg/mL) against above three pathogens. Insilico analysis showed Compound 5 (PTSH) has high affinity towards the Staphylococcus *aureus*, Proteus *mirabilis*, and Enterobacter *cloacae*. In conclusion, chalcone derivatives especially, PTSH is pathogen target specific compound has strongest antimicrobial activity than the commercially chemically

synthesized antibiotics and similarly, our in-house synthesized other compounds also yet to study.

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Author Contributions:
S.Kothai and D.Lakshmi Devi, designed the research work. D.Lakshmi Devi performed the research, analyzed the data and wrote the paper. S.Venkatesh, A.Ganeshkumar and S.Kothai corrected this manuscript. All authors read and approved the final manuscript.
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5. REFERENCES:

[1]. Sivakumar PM, Priya S, Doble M (2009). Synthesis, biological evaluation, mechanism of action and quantitative structure-activity relationship studies of chalcones as antibacterial agents. Chem Biol Drug Des. 73, 403-415.

[2]. Rosenstein IJ, Hamilton-Miller JM, Brumfitt W (1981). Role of urease in the formation of infection stones: comparison of ureases from different sources, Infect. Immun. 32, 32–37.

[3]. Sujoy B, Aparna A (2013).Potential clinical significance of urease enzyme, Europ. Sci.J. Vol.9, No.21.

[4]. Kühler TC, Fryklund J, Bergman N, Weilitz J, Lee A, Akan Larsson H (1995).Structure-activity relationship of omeprazole and analogs as Helicobacter pylori urease inhibitors, J. Med. Chem. 38, 4906–4916.

[5]. Faraci WS, Yang BV, O'Rourke D, Spencer RW (1995). Inhibition of Helicobacter pylori urease by phenyl phosphorodiamidates: mechanism of action, Bioorg. Med. Chem. 3, 605–610.

[6]. Muri EMF, Mishra H, Stein SM, Williamson JS (2004). Molecular modeling, synthesis and biological evaluation of heterocyclic hydroxamic acids designed as Helicobacter pylori urease inhibitors, Lett. Drug Des. Discovery. 1, 30–34.

[7]. Youssef AF, Omar FA, Elsherif HA, Abuo-Rahma GA (1998). Design, synthesis and antidepressant activity of some N-2 substituted Nalidixic acid hydrazides and their cyclized analogs, Bull. Pharm. Sci. Assuit. 21, 15–26.

[8]. Ramadan MA, Tawfik AF, El-Kersh TA, Shibl AM (1995). In vitro activity of subinhibitory concentrations of quinolones on urea-splitting bacteria: effect on urease activity and cell surface hydrophobicity, J. Infect. Dis. 171, 483–486.

[9]. Abdullah MAA, Abd El-Baky R, Hassan HA, Abdelhafez EMN, Abuo-Rahma GA (2016). Fluoroquinolones as urease inhibitors: anti-proteus mirabilis activity and molecular docking studies. Am. J. Microbiol. Res 4,81–84.

[10]. El Zoeiby A, Sanschagrin F, Levesque, RC (2003). Structure and function of the Mur enzymes: development of novel inhibitors. Mol Microbiol. 47,1-12.

[11]. Kahan FM, Kahan JS, Cassidy PJ, Kropp H (1974). The mechanism of action of fosfomycin (phosphonomycin).Ann N Y Acad Sci. 235, 364-386.

[12]. Christensen BG, Leanza WJ, Beattie TR, Patchett AA, Arison BH, Ormond RE, Kuehl FA Jr, Albers-Schonberg G, Jardetzky O (1969). Phosphonomycin: structure and synthesis. Science. 166, 123–125.

[13]. Hendlin D, Stapley EO, Jackson M, Wallick H, Miller AK, Wolf FJ, Miller TW, Chaiet L, Kahan FM, Foltz EL, Woodruff HB, Mata JM, Hernandez S, Mochales S (1969). Phosphonomycin, a new antibiotic produced by strains of streptomyces. Science. 166, 122–123.

[14]. Mendgen T, Scholz T, Klein CD (2010). Structure-activity relationships of tulipalines, tuliposides, and related compounds as inhibitors of MurA. Bioorg Med Chem Lett. 20, 5757-5762.

[15]. Shigetomi K, Shoji K, Mitsuhashi S, Ubukata M (2010). The antibacterial properties of 6-tuliposide B. Synthesis of 6-tuliposide B analogues and structure-activity relationship. Phytochemistry. 71, 312-324.

[16]. Steinbach A, Scheidig AJ, Klein CD(2008). The unusual binding mode of cnicin to the antibacterial target enzyme MurA revealed by X-ray crystallography. J Med Chem. 51, 5143-5147.

[17]. Bachelier A, Mayer R, Klein CD (2006). Sesquiterpene lactones are potent and irreversible inhibitors of the antibacterial target enzyme MurA. Bioorg Med Chem Lett. 16, 5605-5609.

[18]. Han H, Yang Y, Olesen SH, Becker A, Betzi S, Schönbrunn E(2010) . The fungal product terreic acid is a covalent inhibitor of the bacterial cell wall biosynthetic enzyme UDP-N-acetylglucosamine 1-carboxyvinyltransferase (MurA) Biochemistry. 49, 4276-4282.

[19]. Ranjana Pathania A, Eric D Brownb (2008). Small and lethal: searching for new antibacterial compounds with novel modes of action Biochemistry and Cell Biology, 86, 111-115.

[20]. Amy E, Wright, Shirley A, Pomponi Sue S, Cross Peter McCarthy (1992). A new bis-(indole) alkaloid from a deep-water marine sponge of the genus Spongosorites. J. Org. Chem., 57, 4772–4775.

[21]. Shinji Tsujii, Kenneth L Rinehart, Sarath P Gunasekera, Yoel Kashman, Susan S Cross, May S Lui, Shirley A Pomponi, M Cristina Diaz (1988). Topsentin, bromotopsentin, and dihydrodeoxybromotopsentin: antiviral and antitumor bis(indolyl)imidazoles from Caribbean deep-sea sponges of the family Halichondriidae. Structural and synthetic studies. J. Org. Chem. 53, 5446–5453.

[22]. Shinichi Sakemi, Hao H Sun (1991). Nortopsentins A, B, and C. Cytotoxic and antifungal imidazolediylbis [indoles] from the sponge Spongosorites ruetzleri. J. Org. Chem. 56, 4304–4307.

[23]. Jongheon Shin, Youngwan Seo, Ki Woong Cho, Jung-Rae Rho, Chung JSim(1999). New Bis(Indole) Alkaloids of

the Topsentin Class from the Sponge Spongosorites genitrix. J. Nat. Prod. 62, 647–649.

[24]. Sandra A, Morris Raymond, J Andersen (1990). Brominated bis(indole) alkaloids from the marine sponge hexadella SP. Tetrahedron Vol. 46, Issue 3, pp 715-720.

[25]. Ki-Bong Oh, Woongchon Mar, Sanghee Kim, Ji-Yun Kim, Tae-Hoon Lee, Jae-Gyu Kim, Daehyun Shin, Chung J Sim, Jongheon Shin (2006). Antimicrobial Activity and Cytotoxicity of Bis(indole) Alkaloids from the Sponge Spongosorites sp. Biol. Pharm. Bull. 29, 570—573.

[26]. Leena Gupta, Archna Talwar, Prem MS, Chauhan (2007). Bis and Tris Indole Alkaloids from Marine Organisms: New Leads for Drug Discovery. Current Medicinal Chemistry, Vol. 14, Issue 16.

[27]. David W Phife, Robert A Ramos, Ming Feng, Ivan King, Sarath P, Gunasekera Amy Wright, MaheshPatel, Jonathan A Pachter, Stephen J Coval (1996). Marine sponge bis(indole) alkaloids that displace ligand binding to α1 adrenergic receptors. Bioorganic & Medicinal Chemistry Letters. Volume 6, Issue 17, pp 2103-2106.

[28]. Lakshmi Devi D, Reuben Jonathan, Kothai S (2016). Biocidal efficacy of synthetic copolyesters having chalcone moiety. Journal of Chemical and Pharmaceutical Research. 8, 1041-1046.

[29]. Prescott LMH, Arley JP, Klein D (2002). Microbiology (International Edition) Fifth edition. Mc Graw Hill Book Company; pp. 809–819.

[30]. Mitsuru Ueda,Takayoshi Yokoo, Mitsuo Oda (1994). Synthesis of poly(chalcone)s by polycondensation of aromatic dialdehydes with aromatic diacetyl compounds. Volume195, Issue7 Pages 2569-2577.

[31]. Irobi ON, Moo-Young M, Daramola SO (1996). Antimicrobial Activity of Annatto (Bixa orellana) Extract. In J Pharmacognosy. 34, 87–90.

[32]. Wiegand I, Hilpert K, Hancock RE (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 3, 163-75.

[33]. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. 46, 3-26.

[34]. Morris GM, Huey R, Olson AJ (2008). Using AutoDock for ligand-receptor docking. Curr Protoc Bioinformatics, Chapter 8, Unit 8.14.

[35]. BIOvIA D S (2015). Discovery studio modeling environment. Dassault Systemes, San Diego,USA, Release, 4.

[36]. DeLano WL (2002). Pymol: An open-source molecular graphics tool. CCP4. Newsletter On Protein Crystallography. 40, 82-92.

[37]. Daina A, Michielin O, Zoete V (2017). Swiss ADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports.7,42717.

[38]. Patti JM, Allen BL, McGavin MJ, Höök M (1994). MSCRAMM-mediated adherence of microorganisms to host tissues. Annu Rev Microbiol. 48, 585-617.

[39]. Fitzgerald JR, Loughman A, Keane F, Brennan M, Knobel M, Higgins J, Visai L, Speziale P, Cox D, Foster TJ (2006). Fibronectin-binding proteins of Staphylococcus aureus mediate activation of human platelets via fibrinogen and fibronectin bridges to integrin GPIIb/IIIa and IgG binding to the FcgammaRIIa receptor. Mol Microbiol. 59, 212-230.

[40]. Salgado-Pabón W, Breshears L, Spaulding AR, Merriman JA, Stach CS, Horswill AR, Peterson ML, Schlievert PM (2013). Superantigens are critical for Staphylococcus aureus Infective endocarditis, sepsis, and acute kidney injury. MBio. 4(4).

[41]. Lee YT, Lin JC, Wang NC, Peng MY, Chang FY (2007). Necrotizing fasciitis in a medical center in northern Taiwan: emergence of methicillin-resistant Staphylococcus aureus in the community. J Microbiol Immunol Infect. 40, 335-41.

[42]. Martínez-Aguilar G, Avalos-Mishaan A, Hulten K, Hammerman W, Mason, EO, Jr, Kaplan SL (2004). Community-acquired, methicillin-resistant and methicillin-susceptible Stahylococcus aureus musculoskeletal infections in children. Pediatr Infect Dis J. 23, 701-6.

[43]. Hoffmann H, Stindl S, Ludwig W, Stumpf A, Mehlen A, Heesemann J, Monget D, Schleifer KH, Roggenkamp A (2005). Reassignment of enterobacter dissolvens to Enterobacter cloacae as E. cloacae subspecies dissolvens comb. nov. and emended description of Enterobacter asburiae and Enterobacter kobei. Syst Appl Microbiol. 28,196-205.

[44]. Sanders WE Jr, Sanders CC. Enterobacter spp, pathogens poised to flourish at the turn of the century. Clin Microbiol Rev. 1997; 10, 220-241.

[45]. Flynn DM, Weinstein RA, Nathan C, Gaston MA, Kabins SA (1987). Patients' endogenous flora as the source of "nosocomial" Enterobacter in cardiac surgery. J Infect Dis. 156, 363-368.

[46]. Gaston MA (1988). Enterobacter: an emerging nosocomial pathogen. J Hosp Infect. 11, 197-208.

[47]. Sanders CC, Sanders WE Jr (1983). Emergence of resistance during therapy with the newer beta-lactam antibiotics: role of inducible beta-lactamases and implications for the future. Rev Infect Dis. 5,639-648.

[48]. Then RL (1987). Ability of newer beta-lactam antibiotics to induce beta-lactamase production in Enterobacter cloacae. Eur J Clin Microbiol. 6, 451-455.

[49]. O'Hara CM, Brenner FW, Miller JM (2000). Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. Clin Microbiol Rev. 13, 534-46.

[50.Matthews SJ, Lancaster JW (2011). Urinary tract infections in the elderly population. Am J Geriatr Pharmacother. 9, 286-309.

[51]. Papazafiropoulou A, Daniil I, Sotiropoulos A, Balampani E, Kokolaki A, Bousboulas S, Konstantopoulou S, Skliros E, Petropoulou D, Pappas S (2010). Prevalence of asymptomatic bacteriuria in type 2 diabetic subjects with and without microalbuminuria. BMC Res Notes. 3,169.

[52]. Jacobsen SM, Stickler DJ, Mobley HL, Shirtliff ME (2008). Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. Clin Microbiol Rev. 21, 26-59.

[53]. Allison C, Coleman N, Jones PL, Hughes C (1992). Ability of Proteus mirabilis to invade human urothelial cells is coupled to motility and swarming differentiation. Infect Immun. 60, 4740-4746.