Computer-aided Drug Design of New Tripeptides as Inhibitors of *Mycobacterium Tuberculosis* Protein Tyrosine Phosphatase

Kandasamy Nagarajan[1], Anjali Saini[1], Tanya Goyal[1], Parul Grover[1], Garima Kapoor[1]*, Roma Ghai[1], Richa Goel[1], Vinay Kumar[1], Vidhu Saxena[1], Dolly Chauhan[1]

[1] *KIET School of Pharmacy, KIET Group of Institutions, Ghaziabad, Uttar Pradesh, India.*

*Author for correspondence
Dr. Garima Kapoor
Address: KIET School of Pharmacy, KIET Group of Institutions, 13 km milestone, Ghaziabad (U.P), 201206, India
Phone: +91-9643503380
E-mail addresses: kapoor27garima@gmail.com, rknagaraaja@gmail.com

Abstract

Tuberculosis is ubiquitous, 13th underlying cause of death & second major communicable disease after COVID-19. Antibiotic resistance is chief obstruction in treatment of "Mycobacterium tuberculosis." To accomplish prevention from extensively drug resistant & multi drug resistant TB peptides with least side effects, high potency against Mycobacterium protein tyrosine phosphatase-B (mPTPB) is in demand, which is chosen as a target for our study. mPTPB is pivotal for Mtb intracellular survival resulting in decreased macrophage apoptotic activity, demolition of innate immune response which leads to reduced secretion of inflammatory cytokines IL-6. Our aim is to study detailed docking-based investigations of tripeptides against mPTPB as potent inhibitors with more negative docking score based on ΔG value in comparison with standard *cefsulodin. Through preliminary drug like filters of Lipinski rules and Molinspiration, five tripeptides (Pro-Arg-Cys, Arg-Cys-Lys, Arg-Cys-Gly, Arg-Cys-Ala, Arg-Cys-Met) were selected out of 117 tripeptides. Swiss DOCK online tool was used for docking using mPTPB as target (PDB code: 1c83) with HP Laptop having Intel core i5 processor with 8GB Ram and 64-bit operating system. Among the results obtained, Arg-Cys-Gln was found to be the most promising lead as a potent mPTPB inhibitor with ΔG value of -9.46 KCal/mol in comparison to cefsulodin (ΔG value = -11.54 KCal/mol) followed by Arg-Cys-Ile (ΔG value = -8.95 KCal/mol), Arg-Cys-Ala (ΔG value = -8.86 KCal/mol), Pro-Cys-His (ΔG value = -8.82 KCal/mol), Pro-Arg-cys (ΔG value = -8.61 KCal/mol). These results indicate that Arg-Cys-Ile can be considered as a good therapeutic agent against mPTPB rather than the first highest docking score compound as Arg-Cys-Gln has permeability glycoprotein substrate specificity.*

Keywords: *Tripeptides, Docking, Protein Tyrosine Phosphatase B (mPTPB)*

1. Introduction

Tuberculosis is a catching disease caused by endo cellular pathogen Mycobacterium tuberculosis. According to Tuberculosis report given by WHO in 2021, tuberculosis is the second leading cause of death following COVID-19 & about one fourth of the world's population is infected with M. tuberculosis. There are around 50 antibiotics currently available in the clinical pipeline yet the chief obstruction in tuberculosis treatment, that is antibiotic resistance cannot recommend by pharmaceutical industries.

Protein tyrosine phosphatases (PTPs) are signaling enzymes that actuate dephosphorylation of phosphorylated tyrosine residues on protein. Contrary nature of protein tyrosine kinases, protein tyrosine phosphatases conjointly control cell signaling pathway, cell proliferation and other cellular activities. Following ingression in host cell, bacteria like Mycobacterium tuberculosis unfold distinct strategies to evade host signaling process for its survival & replication in host. Mycobacterium tuberculosis encrypt for two PTPs, Mycobacterium protein tyrosine phosphatase A (mPTPA; RV2234) and Mycobacterium protein tyrosine phosphatase B (mPTPB; RV0153c) secreted in host cell's cytoplasm by bacteria. Both phosphatases effect host signaling to evade host's antimicrobial functions.

As a result, mPTPA and mPTPB are promising targets for anti-TB medication development. Macrophages reduce inflammatory cytokine output and limit macrophage death after Mtb infection [1], indicating that both mPTPA and mPTPB are required for Mtb intracellular survival. mPTPB also helps macrophages live longer by elevating Akt phosphorylation and inhibiting caspase-3 activation [2]. All the study results, point to mPTPB messing with innate defence systems by inhibiting bactericidal immune function and boosting macrophage existence.

Charlotte et al., demonstrated significant in vitro activity of an antitubercular peptide comprising proline-arginine rich amino acid in 2001 [3]. Chen et al., discovered that mPTPB is a crucial virulence factor for Mtb sustenance in host macrophages by scanning many compounds against it and recognising numerous 2-oxo-1,2-dihydrobenzo[cd]indole-6-sulfonamide and piperazinyl-thiophenyl-ethyl-oxalamide derivatives as novel mPTPB inhibitors in 2010 [4].

In 2013, He et al., investigated a benzofuran salicylic acid scaffold as a very effective and specific mPTPB inhibitor with an IC50 of 38 nM [5]. In 2014, He et al., developed a salicylic acid-based, drug as mPTPB inhibitor with an IC50 of 2 mM for TB treatment [6]. In 2015, He et al., discovered that -sulfophenyl acetic amide (SPAA) from the third-generation -lactam cephalosporin antibiotic cefsulodin was the most powerful and specific mPTPB inhibitor, with a ki value of 7.9 nM and a ligand efficiency of 0.46 [7].

In 2016, Chen et al., discovered that bostrycin and one of its derivatives were effective inhibitors of mPTPB (IC50 = 327.6 mM and 64.6 mM, respectively) [8]. In 2021, Chen et al., found fusarielins M, N, and G in the marine-derived fungus Fusarium graminearum, and selected fusarielins M as being the most effective molecule targeting mPTPB, with an IC50 of 1050.08 M and a inhibition constant (Ki) of 1030.39 M [9].

The potency of plectasin as an antifungal peptide against Mycobacterium species was revealed by Erik et al., in 2018 [10]. By a structure-based virtual screening technique, Zhang et al., found a thiobarbiturate-based medication as mPTPB non-competitive inhibitor

with a Ki of 2.47 mM and an IC50 of 22.4 mM in 2019 [11]. Shamima et al., stated in 2019 that amino acids can be considered to make peptides that can be used to forecast, diagnose, and cure tuberculosis [12].

Our goal is to use a rigorous docking study technique to build a short chain peptide lead, preferably a tripeptide, as powerful inhibitors of Mycobacterium TB protein tyrosine phosphatase B.

2. Materials and Methods

The system configuration in which the docking study and other online drug design tools were performed ran at 1.80 GHz. Hewlett Packard laptop with Intel ® Core TM processor, 8 GB RAM, and Windows 64-Bit operating system. Based on thorough literature studies [13], one hundred seventeen smaller chain tripeptide molecules were designed. These were taken for the study and subjected to SWISS ADME online to check the drug likeliness property of Lipinski rule of five along with pharmacokinetics. Bioavailability of all compounds were tested against permeability glycoprotein (PGP) substrate along with metabolic profile of drug against various cytochrome P450 inhibition (CYP1 A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4).

For all 117 tripeptides molecule, the Molinspiration cheminformatics online tool was used to estimate different physicochemical properties as well as a bioactivity score on enzyme inhibition as a usual methodology of finding the most appropriate powerful molecule [14]. The selective tripeptides were docked using the online free Swiss Dock tool [15] based on early findings obtained with a target protein, protein tyrosine phosphatase B (mPTPB), which were retrieved sequentially from zinc database I.D and PDB code (1C83). Cefsulodin is the standard medication used to compare docking with the evaluated tripeptides. Biovia Discovery studio visualizer was used to further visualize and analyze bonding interactions in 3D and 2D. In silico toxicity evaluation was carried out using an online server ProTox-II that gives predicted oral toxicity, carcinogenicity, and hepatotoxicity values for designed molecules. Bowman index is also calculated online, to predict the binding potential of the drug as best antitubercular lead.

3. Results:

The Swiss ADME tool was used to analyze physicochemical properties (molecular weight, number of heavy atoms, number of aromatic heavy atoms, number of rotatable bonds, number of hydrogen bond donors, number of hydrogen bond acceptors, molar refractivity, total polar surface area), lipophilicity (consensus logP o/w), water solubility, all ADME properties (GI absorption, blood brain barrier permeation, p-gp substrate, cytochrome P450 inhibitors-CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4, skin permeation (log kp), Lipinski rule of drug likeness characteristics, bioavailability score, lead likeness and synthetic accessibility of all 117 smaller chain tripeptides (log S) were done. Table 1 shows the best 13 tripeptides based on different properties and the findings.

The same collection of chemicals of 117 designed molecules were also run with Molinspiration cheminformatics tools, with the results shown in Table 2 of best 13 tripeptides which were screened out as general enzyme inhibitors using the relative

bioactivity score, viz Pro-cys-his, Arg-cys-met, Arg-cys-gly, Arg-cys-leu, Arg-cys-ala, Pro-his-lys, Pro-arg-cys, Arg-ala-cys, Arg-cys-val, Arg-cys-ile, Arg-cys-gln, Pro-met-his and Pro-asp-cys.

Furthermore, the PASS online way2drug online software tool was employed for all, compounds with their isomeric SMILES notation to evaluate the additional bioactivity of tripeptides against a specific target for TB with probable adverse and toxic effects prediction. Table 3 shows the results of the PASS activity value.

13 chosen Tripeptides undergo docking exploration notably Pro-Cys-His, Arg-Cys-Gly, Arg-Cys-Ala, Arg-Cys-Met, Pro-Arg-Cys, Arg-Cys-Lys, Pro-His-Lys, Ala-Arg-Cys, Pro-Asp-Cys, Pro-Met-His, Arg-Cys-Val, Arg-Cys-Ile, Arg-cys-lys, against the target mPTPB (PDB Code: 1c83) using cefsulodin as the reference drug. Table 4 shows the results of the docking investigation with ΔG value (Kcal/mol). Table 4 lists the results of the extensive docking analysis of all investigated compounds Pro-Cys-His, Arg-Cys-Gly, Arg-Cys-Ala, Arg-Cys-Met, Pro-Arg-Cys, Arg-Cys-Lys, Pro-His-Lys, Ala-Arg-Cys, Pro-Asp-Cys, Pro-Met-His, Arg-Cys-Val, Arg-Cys-Ile, Arg-cys-lys, and the best molecular docking contacts are clearly depicted in Figures 1-5.

Arg-Cys-Gln was determined to be the most propitious molecule as a powerful mPTPB inhibitor, with a ΔG value of -9.46 kcal/mol, out of the Thirteen tripeptide frameworks developed (Pro-Cys-His, Arg-Cys-Gly, Arg-Cys-Ala, Arg-Cys-Met, Pro-Arg-Cys, Arg-Cys-Lys, Pro-His-Lys, Ala-Arg-Cys, Pro-Asp-Cys, Pro-Met-His, Arg-Cys-Val, Arg-Cys-Ile, Arg-cys-lys,).

The detailed docking investigation of 8 tripeptides with best docking score and cefsulodin standard are tabulated in Table 5, and their best molecules docking interactions are clearly visualized in Figures 1-5. Among the thirteen tripeptide templates (Pro-Cys-His, Arg-Cys-Met, Arg-Cys-Gly, Arg-Cys-Leu, Arg-Cys-Ala, Pro-His-Lys, Pro-Arg-Cys, Arg-Ala-Cys, Arg-Cys-Val, Arg-Cys-Ile, Arg-Cys-Gln, Pro-Met-His, Pro-Asp-Cys) obtained, Arg-Cys-Gln was found to be the most promising compound as potent mPTPB inhibitor with ΔG value of -9.46 kcal/mol in comparision to the standard drug cefsulodin having ΔG value of -11.54 kcal/mol (full energy = -1773.45 kcal/mol) followed by Arg-Cys-Ile (ΔG value of -8.95 kcal/mol) and Arg-Cys-Ala (ΔG value of -8.86 kcal/mol) respectively. In addition, the tripeptide Arg-Cys-Gln showed good conventional hydrogen bond interaction with the bond distance as 2.88 A° and 2.89 A° (A: Ser 216:N-A:OAI301:016, A: Arg 216:N-A:OAI301:016) as compared to cefsulodin with best bond distances as 2.62 A° and 2.01 A° respectively (Arg 254: HH 11; Gly 259: HN-). The results of cefsulodin's conventional hydrogen bond have extra features with respective bond distance as 2.69 A° and 2.94 A° (Arg 24: HH 21; Gln 262: HE 22), clearly indicated that the interaction with arginine and glutamine site of mPTPB is attributed to best therapeutic potency of antitubercular drug. As the hydrogen bond interaction indicates a more stable complex between the chosen tripeptide & protein target, all the observed interactions of tripeptide Arg-Cys-Gln is less than the ideal bond distance (3 A° & less).

The most promising tripeptide lead Arg-Cys-Gln contain only two hysrogen bond interation, one at Ser216 site and the other at Arg 216 site, which shows that 216 pocket is very essential for activity, where as the standard cefsulodin have shown Arg 254 site is very essential for higher potency in addition to Gln 262, Gly 259 Arg 24 site.

The second most potent lead Arg-Cys-Ile has shown 2 conventional hydrogen bond interaction one at Arg 221 and other at Ala 217 site, which again signifies a Arg 221 pocket is crucial for activity as like test drug Arg-Cys-Gln (Arg 216 pocket) and Cefsulodin (Arg 254 pocket).

Other test drugs like Arg-Cys-Ala also shown 6 hydrogen bond interaction, in which Gln 102 site interaction (3.04 A°) is crucial as like standard cefsulodin with Gln 262 site (2.94 A°). Similarly other tripeptide leads like Pro-Cys-His and Pro-Arg-Cys also shown 3 and 4 hydrogen interactions with serine residue is important as Ser216 pocket interaction (2.88 A°) in the former and Ser 80 pocket interaction (2.55 A°) in the later lead, which clearly indicates Proline containing amino acid sequence in tripeptide lead essentially needs Serine pocket for crucial hydrogen bonding interaction.

The toxicity prediction of all eight tripeptide leads using Pro-Tox-II software for most of the potent tripeptide leads with predicted LD50 (mg/kg) as 5000mg/kg (Toxicity class-5) except Pro-Cys-His with LD50 (mg/kg) as 3000 mg/kg (Toxicity class-5) respectively.

In addition, the protein binding potential of tripeptide leads was calculated with Boman index (kcal/mol), which shows that the tripeptide Arg-Cys-Ala has 9.65kcal/mol as low value, indicates its antimicrobial potential with less side effects, whereas the other tripeptide leads, especially Proline containing tripeptide leads, Pro-Cys-His and Pro-Arg-Cys has the boman index of 2.54kcal/mol & 3.6kcal/mol, which signifies a higher value more than 2.48, these indicating a prominent multifunctional role of peptide for therapeutic ailment of tuberculosis.

Table 1. Swiss ADME Physicochemical characteristic features of smaller chain Tripeptides

S.No.	Tri-peptides	Pharmacokinetic Properties									Drug Likeness			
		GI Ab	BBB Pe	PGP S	CYP 1A	CYP 2C	CYP 2	CYP 2D	CYP 3	Log K_p Permea	Lipins	Bio-ava Score	Lead	Synth Acces
1	Pro-Arg-Cys	Low	No	No	No	No	No	No	No	-10.34	Yes	0.55	No	3.99
2	Pro-His-Lys	Low	No	No	No	No	No	No	No	-10.99	Yes	0.55	No	4.04
3	Pro-Met-His	Low	No	No	No	No	No	No	No	-10.39	Yes	0.55	No	4.05
4	Pro-Asp-Cys	Low	No	No	No	No	No	No	No	-12.05	Yes	0.11	No	3.59
5	Arg-Cys-Gln	Low	No	Yes	No	No	No	No	No	-12.99	No	0.17	No	4.14
6	Arg-Cys-Gly	Low	No	No	No	No	No	No	No	-12.02	No	0.55	No	3.56
7	Arg-Ala-Cys	Low	No	No	No	No	No	No	No	-11.82	Yes	0.55	No	3.84
8	Arg-Cys-Ala	Low	No	No	No	No	No	No	No	-11.82	Yes	0.55	No	3.85
9	Pro-Cys-His	Low	No	No	No	No	No	No	No	-10.73	Yes	0.55	No	3.74
10	Arg-Cys-Leu	Low	No	No	No	No	No	No	No	-11.45	Yes	0.55	No	4.16
11	Arg-Cys-Met	Low	No	No	No	No	No	No	No	-11.73	Yes	0.55	No	4.28
12	Arg-Cys-Val	Low	No	No	No	No	No	No	No	-11.31	Yes	0.55	No	4.06
13	Arg-Cys-Ile	Low	No	No	No	No	No	No	No	-11.20	Yes	0.55	No	4.31
14	Cefsulodin (S	Low	No	No	No	No	No	No	No	-9.88	No	0.11	No	5.04

Table 2: MOLINSPIRATION FEATURES WITH BIOACTIVITY SCORE OF SMALLER CHAIN TRIPEPTIDES

S. No.	**Tripeptides**	Enzyme Inhibitors Bioact Score
1	Pro-Arg-Cys	0.72
2	Pro-His-Lys	0.73
3	Pro-Met-His	0.66
4	Pro-Asp-Cys	0.65
5	Arg-Cys-Gln	0.71
6	Arg-Cys-Gly	0.80
7	Arg-Ala-Cys	0.71
8	Arg-Cys-Ala	0.74
9	Pro-Cys-His	0.89
10	Arg-Cys-Leu	0.74
11	Arg-Cys-Met	0.81
12	Arg-Cys-Val	0.70
13	Arg-Cys-Ile	0.70

Table 3: PASS ONLINE BIOACTIVITY SCORE & TOXICITY PREDICTION OF SMALLER CHAIN TRIPEPTIDES

S.No.	Tripeptic	Target for TB	P_A	P_I	Possible Adverse and Toxic Effects
1	Pro-cys-his	Glutamate-5-semialdehyde dehydrogenase inhibitor	0,772	0,019	nil
2	Arg-cys-met	Pyroglutamyl-peptidase I inh	0,870	0,006	nil
3	Arg-cys-gly	Proteasome ATPase inhibito	0,785	0,006	nil
4	Arg-cys-leu	Proteasome ATPase inhibito	0,709	0,013	nil
5	Arg-cys-ala	Membrane integrity antagoni	0,827	0,005	nil
6	Pro-his-lys	Glutamate-5-semialdehyde dehydrogenase inhibitor	0,791	0,017	Hypertension
7	Pro-arg-cys	Proteasome ATPase inhibito	0,891	0,005	nil
8	Arg-ala-cys	Proteasome ATPase inhibito	0,835	0,004	ulcer (may be)
9	Arg-cys-val	Pyroglutamyl-peptidase I inh	0,718	0,012	nil
10	Arg-cys-ile	Pyroglutamyl-peptidase I inh	0,898	0,005	nil
11	Arg-cys-gln	Pyroglutamyl-peptidase I inh	0,884	0,005	nil

12	Pro-met-his	Glutamate-5-semialdehyde dehydrogenase inhibitor	0,744	0,022	nil
13	Pro-asp-cys	Proteosome ATPase inhibito	0,827	0,004	nil

Table 4. Docking Score of tripeptides with ΔG value

S.No.	Tripeptides	delta G value
1	Pro-Cys-His	-8.82
2	Arg-Cys-Met	-8.6
3	Arg-Cys-Gly	-8.33
4	Arg-Cys-Leu	-8.59
5	Arg-Cys-Ala	-8.86
6	Pro-His-Lys	-8.4
7	Pro-Arg-Cys	-8.61
8	Arg-Ala-Cys	-8.58
9	Arg-Cys-Val	-8.25
10	Arg-Cys-Ile	-8.95
11	Arg-Cys-Gln	-9.46
12	Pro-Met-His	-8.27
13	Pro-Asp-Cys	-8.29
14	Cefsulodin	-11.54

Table 5. Conventional Hydrogen bond interactions residues of 8 tripeptides with best docking score

S. N	Tripeptide	Hydrogen bond interactions
1	**Arg-Cys-Ala**	**LIG: LIG1: H1 - : PRO 206 : 0 (2.02 A)** **LIG: LIG1: H22 –: HSD 208 : 0 (1.99 A)** **LIG : LIG1: H3 - : HSD 208 : 0 (1.94 A)** **LIG : LIG1 : H15 - : GLN 102 : 0 (3.04 A)** **LIG : LIG1 : H1 - : LIG : LIG1 : 04 (2.06 A)** **LIG : LIG1 : H16 - :LIG : LIG1 : 04 (2.63 A)**
2	**Arg-Cys-Met**	**LIG : LIG1 :H2 - :SER28 : 0 (1.93 A)** **LIG : LIG1 : H23 - :PHE30 : 0 (2.22 A)**
3	**Arg -Cys-Lys**	**LIG: LIG1:H3 -: ASP 48:0 (2.41 A)** **LIG: LIG1:H1-: ASP 48:0 (2.74 A)** **LIG: LIG1:H15 - LIG: LIG1: 04 (2.19A)** **GLY259:HN – LIG: LIG1 :02 (2.19A)** **LIG: LIG1: H7 -: SER 28 :0 (1.87 A)**
4	**Arg -Cys-Gly**	**LIG: LIG1:H21 -: SER 28:0 (1.99 A)** **GLY259:HN – LIG: LIG1:N6 (2.41A)** **LIG: LIG1: H22- LIG: LIG1:02 (2.74 A)** **LIG: LIG1 H4 -:ASP 48 :0 (3.16 A)**

		LIG : LIG1 : H19 -: SER 28 :0 (2.01 A)
5	**Pro- Arg- Cys**	**LIG: LIG1: H24 -: MET 74:0 (2.72 A)** **LIG: LIG1: H4 -: GLN 78:0 (1.83 A)** **SER 80 : HN – LIG : LIG1 : 03 (2.55 A)** **LIG : LIG1 : H15 -: HSD 208 : 0 (2.46 A)**
6	**Arg-Cys-Gln**	**A: SER 216:N-A:OAI301:016(2.88A),** **A: ARG 216:N-A:OAI301:016(2.89A),**
7	**Arg-Cys-Ile**	**A: ALA 217:N-A:OAI301:016(3.33A),** **A: ARG 221:N-A:OAI301:015(3.07A),**
8	**Pro-Cys-His**	**A: SER 216:N-A:OAI301:016(2.88A),** **A: ARG 221:N-A:OAI301:015(2.78A),** **A: GLY 220:N-A:OAI301:017(2.75A),**
9	**Cefsulodin**	**Arg 254: HH 11 (2.62 A°)** **Arg 24: HH 21 (2.69 A°)** **Gln 262: HE 22 (2.94 A°)** **Gly 259: HN- (2.01 A°)**

Table 6. Predicted LD50 and Toxicity class of the compounds

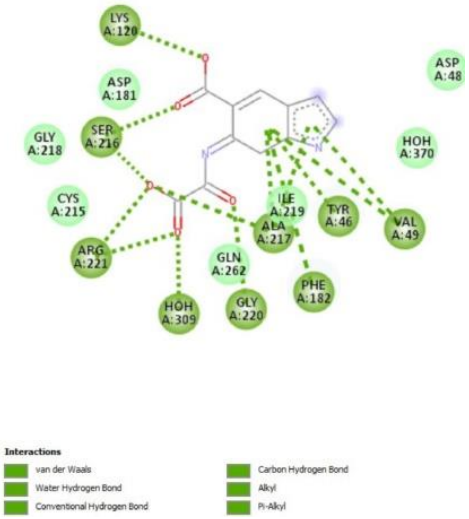
S.No.	Tripeptides	Predicted LD50 (mg/kg)	Predicted toxicity class
1	Arg-cys-ala	5000	5
2	Arg-cys-gly	5000	5
3	Arg-cys-cys	3000	5
4	Arg-cys-met	5000	5
5	Pro-arg-cys	5000	5
6	Arg-cys-gln	5000	5
7	Arg-cys-Ile	5000	5
8	Pro-cys-his	3000	5

Table 7. Bowman Index of the Compounds

S.No.	Tripeptides	Protein-binding Potential (Boman index) kcal/mol
1	Arg-cys-ala	0.65
2	Arg-cys-gly	0.96
3	Arg-cys-cys	1.85
4	Arg-cys-met	2.38
5	Pro-arg-cys	3.66
6	Arg-cys-gln	1.69
7	Arg-cys-Ile	1.26
8	Pro-cys-his	2.54

Figure 1. Docking Investigation of tripeptide Arg-Cys-Gln against the target Protein tyrosine phosphatase. a) 2D Map of ligand's non-bonded interactions b) Conventional pharmacophoric distance measurement of H Bonds between drug and target using in ribbon format Biovia Studio visualizer c) 3D Pocket view of ligand interaction with enzyme.

a)



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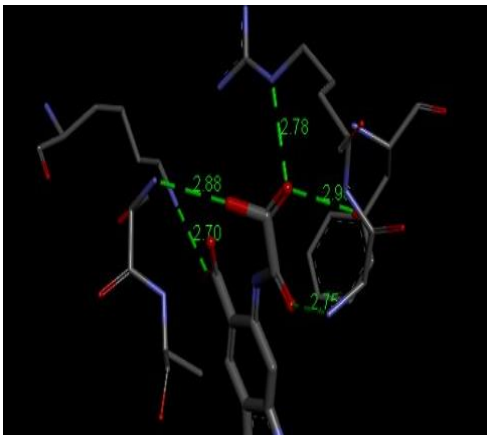

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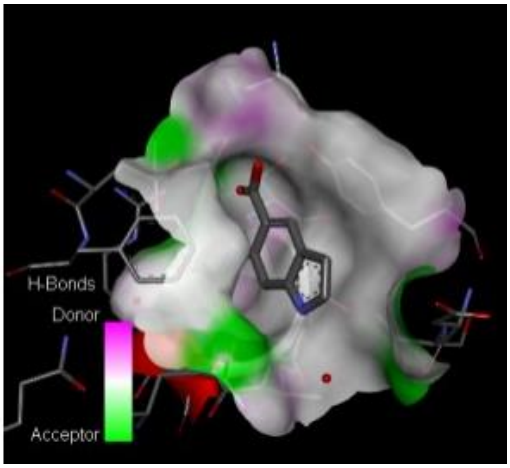
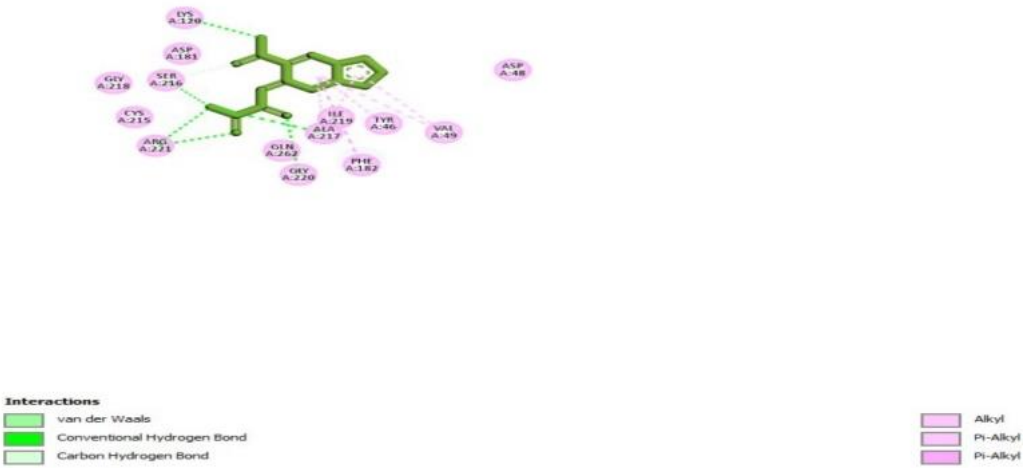


Figure 2: Docking Investigation of tripeptide Arg-Cys-Ile against the target Protein tyrosine phosphatase a) 2D Map of ligand's non-bonded interactions b) Conventional pharmacophoric distance measurement of H Bonds between drug and target using in ribbon format Biovia Studio visualizer c) 3D Pocket view of ligand interaction with enzyme.

a)



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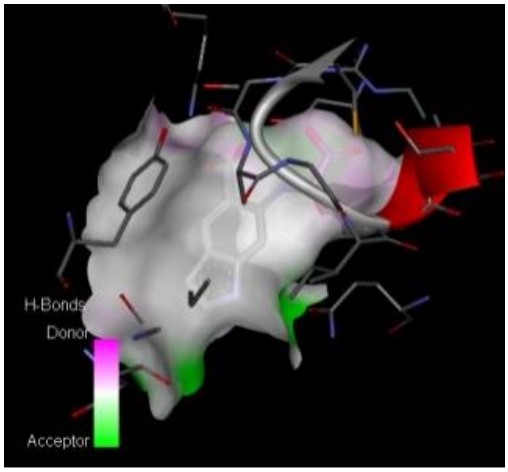

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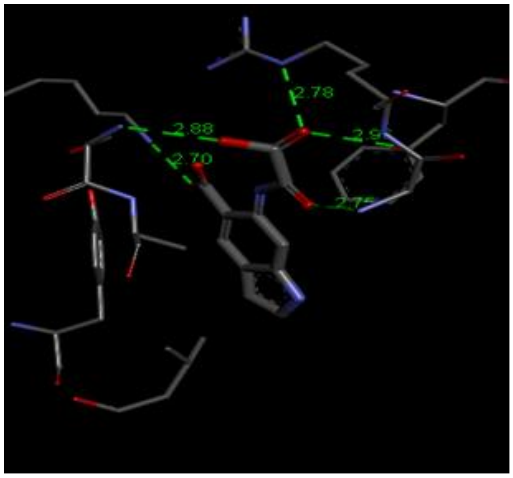
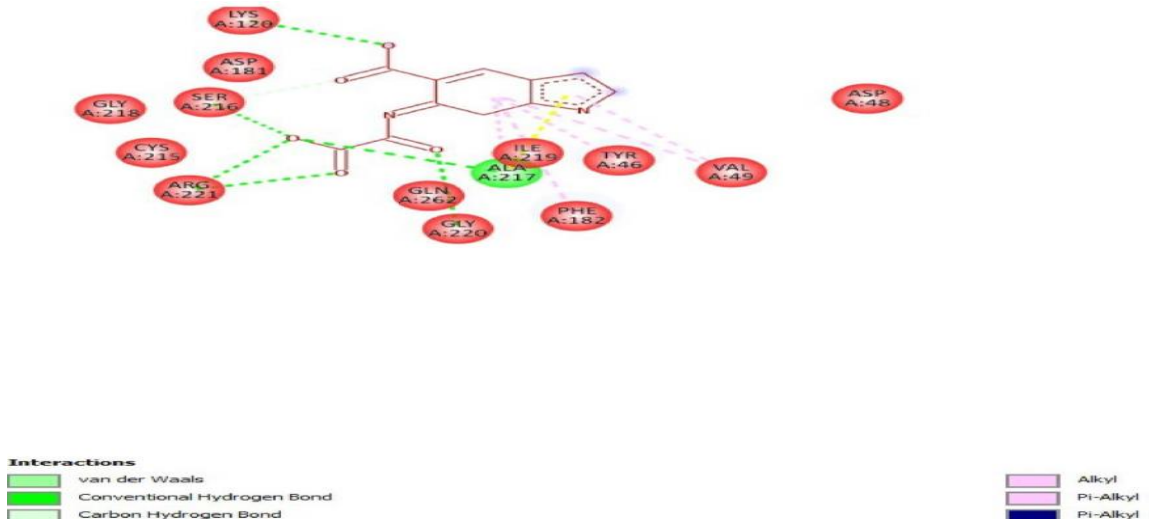


Figure 3: Docking Investigation of tripeptide Arg-Cys-Ala against the target Protein tyrosine phosphatase a) 2D Map of ligand's non-bonded interactions b) Conventional pharmacophoric distance measurement of H Bonds between drug and target using in ribbon format Biovia Studio visualizer c) 3D Pocket view of ligand interaction with enzyme.

a)



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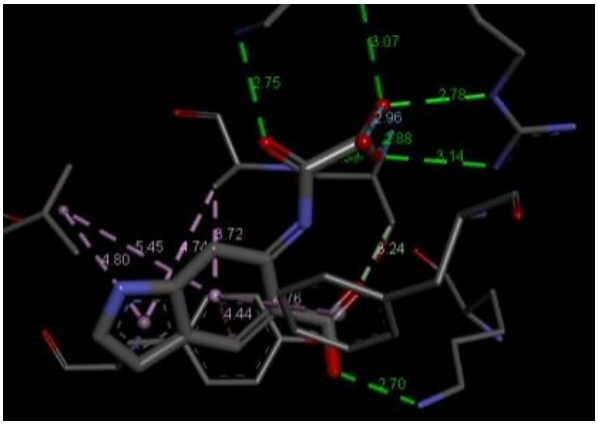


c)

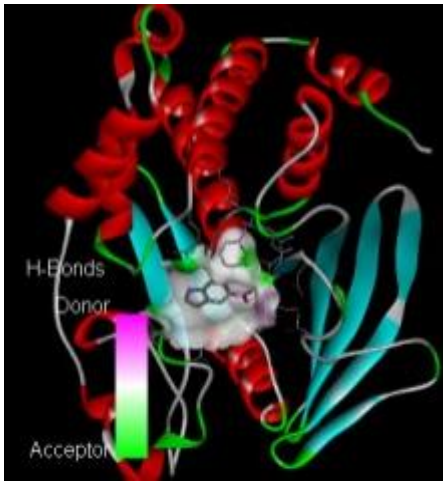
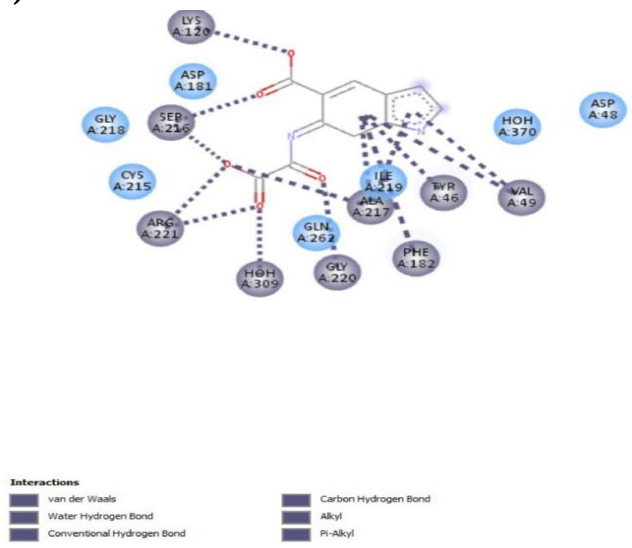
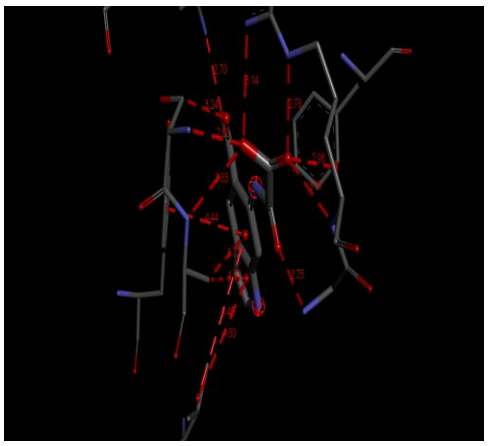


Figure 4: Docking Investigation of tripeptide Pro-Cys-His against the target Protein tyrosine phosphatase a) 2D Map of ligand's non-bonded interactions b) Conventional pharmacophoric distance measurement of H Bonds between drug and target using in ribbon format Biovia Studio visualizer c) 3D Pocket view of ligand interaction with enzyme.

a)



b)



c)

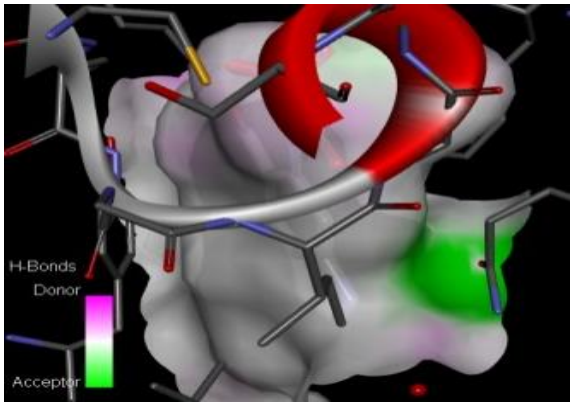
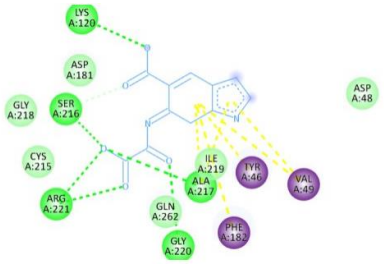
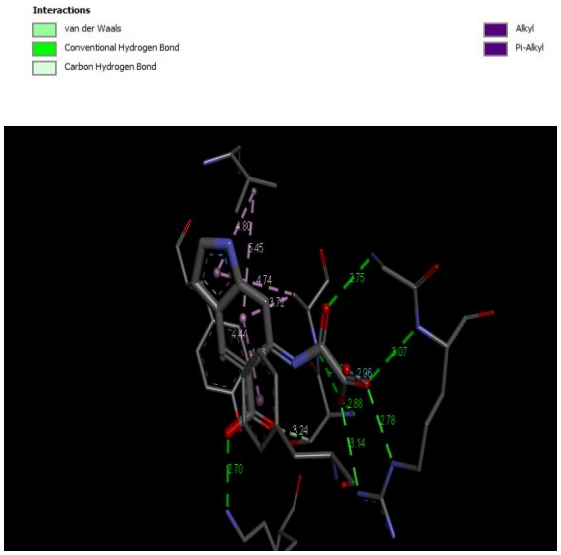


Figure 5: Docking Investigation of tripeptide Pro-Arg-Cys against the target Protein tyrosine phosphatase a) 2D Map of ligand's non-bonded interactions b) Conventional pharmacophoric distance measurement of H Bonds between drug and target using in ribbon format Biovia Studio visualizer c) 3D Pocket view of ligand interaction with enzyme.

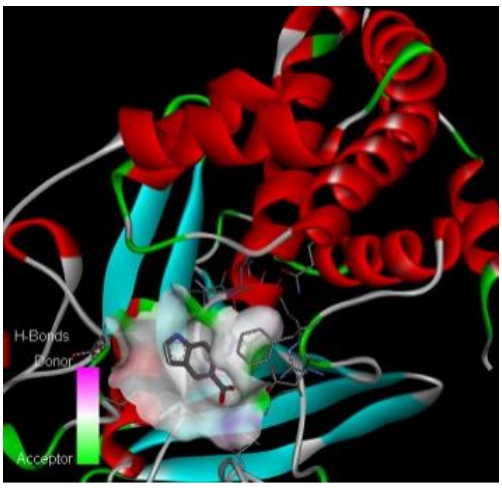
a)



b)



c)



4. Discussion:

Detailed docking investigation identifies the most potent drug as Arg-Cys-Gln with ΔG value as -9.46 Kcal/mol as compared with standard cefsulodin (containing 4 H-bond interactions Arg 254, Arg 24, Gln 262 and Gly 259), our potent drug Arg-Cys-Gln has shown 2 hydrogen bond interactions (Ser 216 and Arg 216).

The binding mode of Arg-Cys-Gln was further explored via molecular docking (Fig. 1c) which suggested that Arg-Cys-Gln binds to the active site of mPTPB, forming a hydrogen bond with the side chain of Arg 216; this is unique in the p-loop of mPTPB and the contact between Arg-Cys-Gln and Arg 216 in the catalytic loop provides a potential basis for inhibitor. Selectivity (bond distance: 1.90 A°), which may not be seen in the standard drug cefsulodin's molecular docking. Therefore, Arg-Cys-Gln shows great potential as an anti-TB drug candidate with respect to detailed docking investigation alone.

As from SWISS ADME pharmacokinetic profile data of Table 1, the most potent drug Arg-Cys-Gln through docking score seems to be identified with permeability glycoprotein as suitable substrate and hence the drug Arg-Cys-Gln may not be chosen further as lead molecule for synthesis, since their efflux is more with reduced bioavailability and probably may fail in clinical phase II stage if chosen as lead. Hence the second most potent lead Arg-Cys-Ile is considered as the best tripeptide lead with ΔG value -8.95 Kcal/mol.

Targeting mPTPB for the treatment of TB offers an alternative strategy to the traditional antibiotic approaches and could provide therapeutic agents that overcome the antibiotic resistance. In practice, the success of a small molecule- based drug discovery project depends on the availability of potent and specific inhibitors with drug-like properties.

Many synthetic PTP inhibitors are based on pTyr mimetic as the central building block [16]. This approach has the advantage that it is relatively easy to find starting points for inhibitors. However, the phosphotyrosine mimetic poses a challenge to introduce sufficient selectivity, because the phosphotyrosine-binding pockets of different PTP enzymes are similar. This problem is reinforced by the observation that isolated PTP catalytic domains often exhibit modest substrate specificity, with cellular localization and the activities of appended substrate-binding domains enhancing selectivity in vivo [17]. This may be the case observed with our smaller chain peptide Arg-Cys-Gln with similar probable mode of action specificity.

Significant advances were made in the mPTPB inhibitor development by Ghattas and co-workers, conducted a druggability assessment on 17 PTPs [18]. Their assessment revealed that only two of 17 PTPs, namely mPTPB and GLEP-1 are likely druggable due to their large hydrophobic active sites.

Due to the presence of aromatic amino acid histidine in Pro-Cys-His and cystine in Pro-Arg-Cys, we may predict that the hydrophobic interaction is possible with dipeptide (His and Cys) and the active site of mPTPB (His). Mycobacterium phosphatases have emerged as key players, especially mPTPB in interfering with the MAPK and AKT signal transduction pathways. The potent tripeptide Arg-Cys-Gln and Arg-Cys-Ile discussed above are not only useful as starting points for the development of therapeutic agents for TB but can also serve as molecular probes in understanding the structure and function of these enzymes in TB pathogenesis. Hence PTPB inhibition by small molecules could impact Mtb

survival in the host and open the way for the development of innovative therapeutic strategies. Particularly, the localization outside of the mycobacterium cell wall, which is difficult to penetrate, renders these enzymes attractive targets with utmost safety, against any resistance type, MDR and XDR tuberculosis. Further in vitro investigation of tuberculosis with automated microbial detection system is necessary for subjecting the potent lead Arg-Cys-Gln and Arg-Cys-Ile after synthesizing the same. Since, Arg-Cys-Gln even though having high docking score but due to permeability glycoprotein substrate specificity, we are eliminating this molecule and the best compound was found to be Arg-Cys-Ile. If we consider this as best potent lead, they may get effluxes due to Pgp substrate specificity with decreased bioavailability inside the cell and automatically get eliminated subsequently in Phase-II clinical trial.

After COVID-19 scenario *in-silico* study are gaining more popularity and to keep abridge with environmental sustainability we have performed *in-silico* study on shorter chain biofriendly peptide leads and come out with best possible lead as anti-tubercular candidates which will further open the horizon to synthesize and perform further biological activity in animal models in near future.

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