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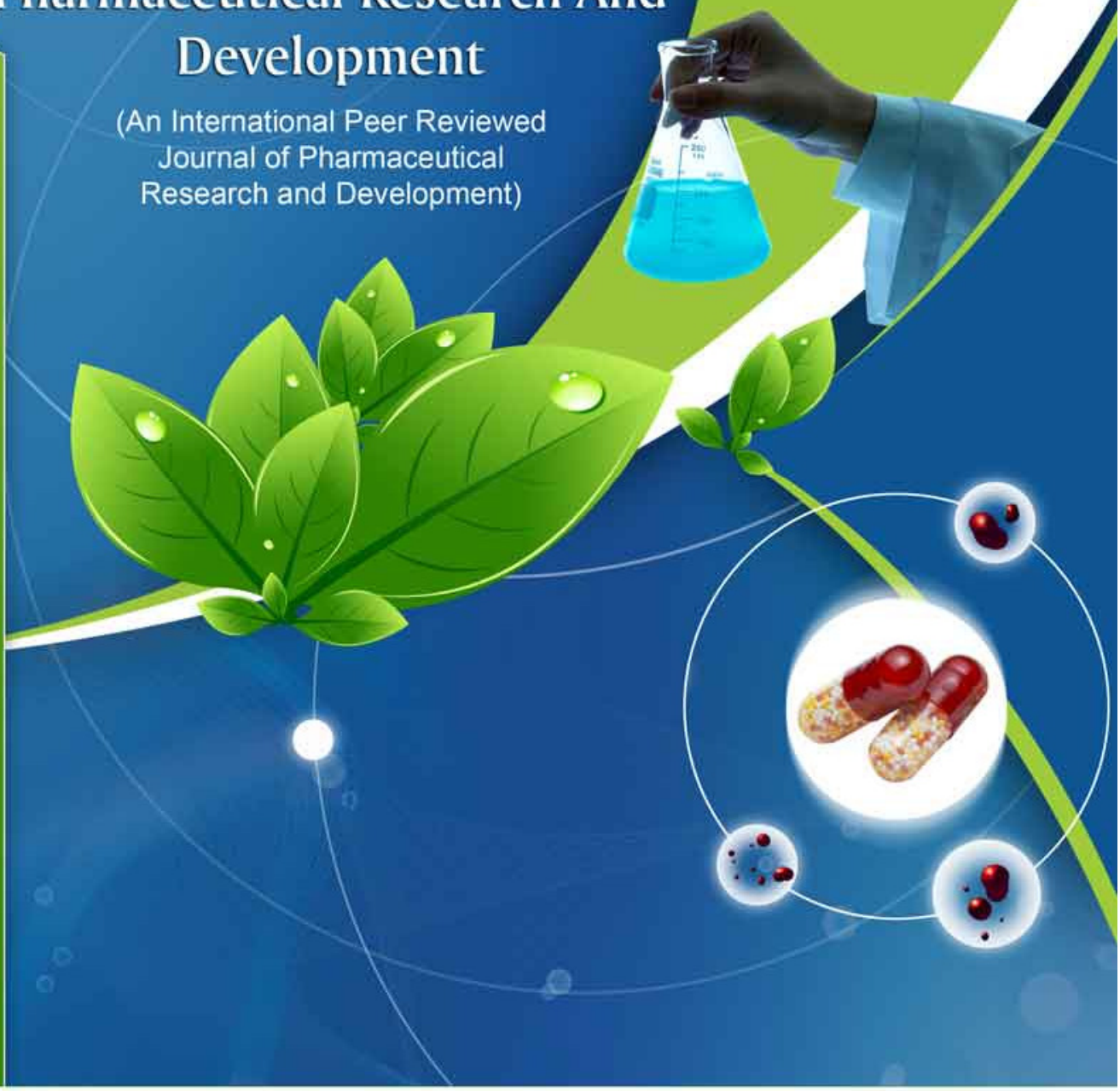

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Research Article

VIRTUAL DOCKING STUDIES OF FLAVONOID COMPOUNDS AGAINST CELL WALL PROTEINS OF *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

Tuberculosis continues to be a major cause of morbidity and mortality throughout the world. Considering the world–wide TB problems, there is an urgent need to develop relatively inexpensive new drugs to treat this deadly disease. The two main avenues of drug discovery are: identifying new microbial proteins for which to direct drug discovery efforts, and designing innovative drugs that target existing proteins. Natural products isolated from plants have played an important role in discovery of drugs against infectious diseases. In this present study, 50 ligand molecules (basically secondary metabolites, flavonoids) which were commonly present in the plants were docked with the selected Mycobacterium tuberculosis receptors (PDB ID- 1DQY, 1KPI and 1TQ8) using iGEMDOCK. Among them, five compounds had a significant inhibitory activity with the receptors at a very low energy value. This was also found to obey the Lipinski's Rule of five and showed the drug likeliness and bioavailability. Since it is from a natural source the compound is non toxic and has reduced side effects.

Keywords: *Cell wall proteins, docking, flavonoids, iGEMDOCK, Mycobacterium tuberculosis.*

INTRODUCTION

Mycobacterium tuberculosis is the etiologic agent of tuberculosis in humans. Tuberculosis (TB), a decimating disease affecting one third of the human population and causing around two million deaths every year according to the World Health Organization [1]. *M. tuberculosis* belongs to the genus Mycobacterium and is a slow-growing, gram positive, aerobic rod-shaped, facultative intracellular pathogen which has the ability to survive and multiply inside macrophages [2, 3]. Tuberculosis, a lung infection and is one of the contagious and deadly diseases which have added to the woes of the mankind.

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Although several antibiotics and the 'Directly Observed Treatment, Short-course' (DOTS) [4] have been used to effectively reduce the burden of TB, emergence of drug resistant and drug-sensitive TB and co-infection with HIV result in increasing incidence of TB in recent years [5]. Therefore, it is crucial to identify novel targets to develop new approaches and agents for anti-drug-resistant and drug-sensitive *M. tuberculosis.* To do this, biochemical pathways specific to the mycobacteria and related organism's disease cycle must be better understood. Many unique metabolic processes occur during the biosynthesis of mycobacterial cell wall components [6]. One of these attractive targets for the rational design of new antitubercular agents are the mycolic acids and the major components of the cell wall of *Mycobacterium tuberculosis* [7].

Mycobacteria have an unusual cell wall in which mycolic acids play a critical role in pathogenesis and persistence. Important characteristics conferred by this structure are resistance to

chemical injury, low permeability to antibiotics, resistance to dehydration, and ability to thrive within the hostile environment of the macrophage phagolysosome [8]. Proteins of the antigen 85 complex are responsible for the high affinity of mycobacteria to fibronectin. Each protein possesses a mycolyltransferase activity required for the biogenesis of trehalose dimycolate (cord factor), a dominant structure necessary for maintaining cell wall integrity [9].

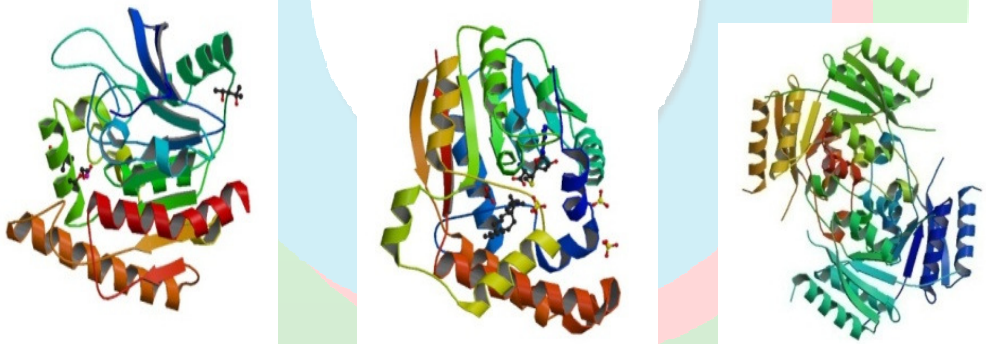
Several studies indicate that functional groups in the acyl chain of mycolic acids are important for pathogenesis and persistence. There are three Mycolic acid cyclopropane synthases (PcaA, CmaA1, and CmaA2) responsible for the site-specific modifications of mycolic acids [10]. Considering the importance of the cell envelope structure for bacterial survival [11,12], many attempts have been made to identify the enzymes involved in the metabolism of such specific compounds which obviously represent attractive targets for the design of new anti mycobacterial drugs.

The discovery of novel drugs to treat diseases is still an important area of pharmaceutical research. Structure-based drug design (SBDD) is one of the most promising ways in this endeavor. It has been shown for a large number of three dimensional (3D) structures of proteins can be used to design small drug molecules that can bind tightly to the active site of protein [13-15]. *In silico* methods are used to analyze the target structures for possible binding sites, generate candidate molecules, check for their drug likeness, dock these molecules with the target, rank them according to their binding affinities, and further optimize the molecules to improve binding characteristics.

MATERIALS AND METHODS

Receptors selected for this study

The three dimensional structure of the target proteins, Antigen 85C complexed with Diethyl phosphate (Figure 1. (A) PDB ID – 1DQY), Mycolic acid cyclopropane synthase CmaA2 complexed with SAH and DDDMAB (Figure.1.(B) PDB ID –1KPI), and a hypothetical protein Rv1636 complexed with MSE (Figure 1.(C) PDB ID –1TQ8) which is present in the cell wall of *Mycobacterium tuberculosis* were obtained from **P**rotein **D**ata **B**ank [16].



A B C

Figure 1: Crystal structure of targets: PDB ID (A) 1DQY (B) 1KPI (C) 1TQ8

Preparation of ligand library

Chemical structures were retrieved from ZINC database [17]. The set of ligand molecules selected for this study were 50 flavonoids compounds from different plant sources. Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure [18]. Over 6,500 flavonoids have been identified, many of which occur in fruits, vegetables and beverages [19]. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health-they have been reported to have anti-microbial, anti-allergic, anti-platelet, anti-inflammatory, anti-tumor and anti-oxidant activities [20-22]. These phytochemicals were screened *in silico* for their inhibitory activity against the selected protein molecules. The structure of these compounds is shown in Figure 2.

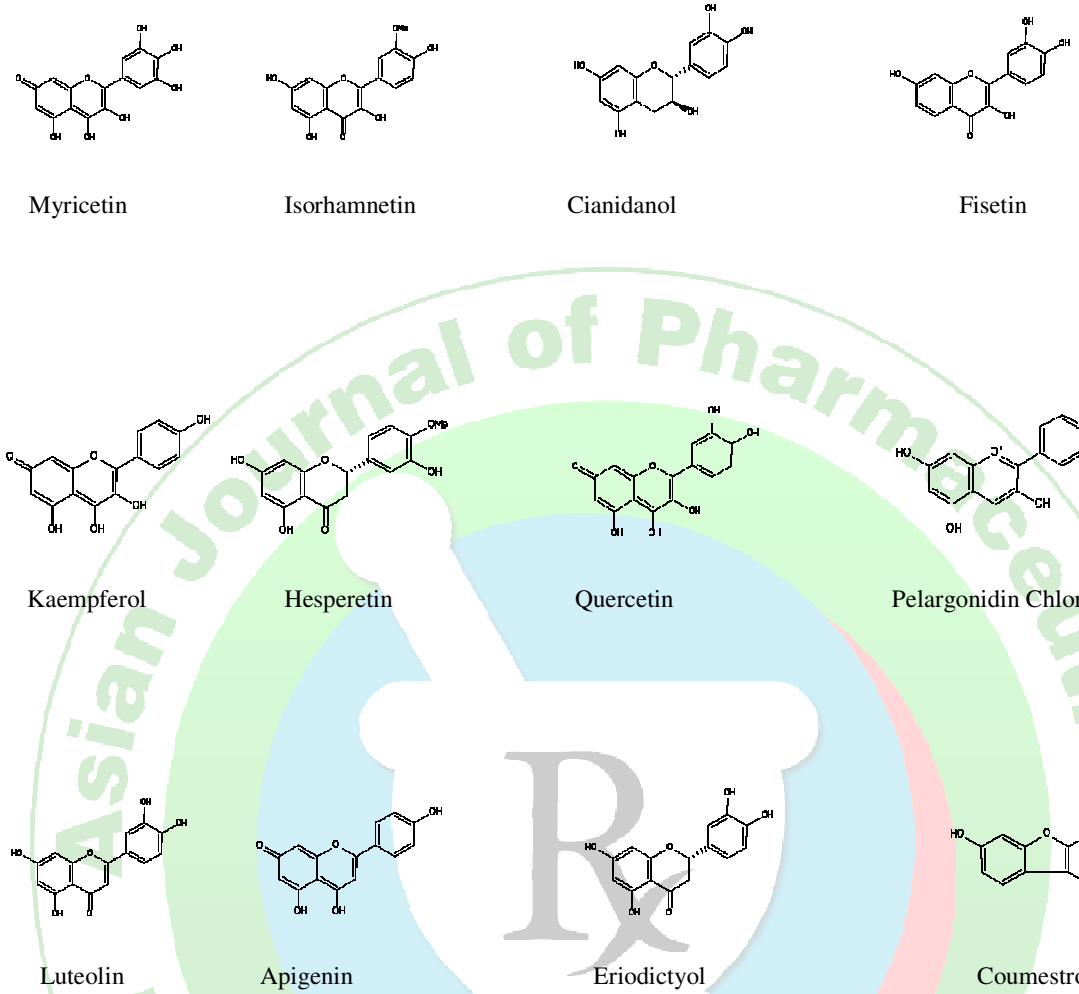


Figure 2: Structure of screened flavonoid compounds used in this study

Docking and Screening

In order to carry out the docking simulation, we used the *i*GEMDOCKv [23] as molecular docking tool. *i*GEMDOCKv is an integrated Virtual Screening environment from pre-preparation through post-screening analysis with pharmacological interactions. The docking protocol consisted of 70 generations per ligand and the population size of 200 random individuals. All the docking conformations were performed twice using genetic evolutionary algorithm and the fitness of the docked structures were calculated. The hydrophobic preference and electrostatic preference were set to 1.00. The binding site of the target was identified at a distance 8Å.

The empirical scoring function of *i*GEMDOCK was estimated as:

Fitness = vdW + Hbond + Elec.

Here, the vdW term is van der Waal energy. Hbond and Elect terms are hydrogen bonding energy and electro statistic energy, respectively.

The pharmacological scoring function of *i*GEMDOCK was estimated as

$E_{pharma}=E_{GEMDOCK}+E$ (E) $_{pharma}+2E$ (H) $_{pharma}+0.5$E (V) $_{pharma}$

Where $E_{GEMDOCK}$ is the docked energy of GEMDOCK and E (E) $_{pharma}$, E (H) $_{pharma}$ and E (V) $_{pharma}$ are the pharmacological scores of electrostatic, hydrogen bonding and vdW interactions, respectively.

Based on these profiles and compound structure, *i*GEMDOCK infers the pharmacological interactions and clusters the screening compounds for the post screening analysis. Finally *i*GEMDOCK ranks and visualizes the screening compounds by combining the pharmacological

interactions and energy- based scoring functions of *i*GEMDOCK.

RESULTS AND DISCUSSION

Docking of small chemical compounds onto the binding site of a receptor and estimating the binding energy of the complex is an important part of Structure Based Drug Discovery (SBDD). In this present study, selected three receptor protein molecules were docked with 50 flavonoid compounds. These ligands were screened for their ability to dock within the receptor molecule and to find those chemical compounds which can inhibit the activity of the protein. In order to enhance the accuracy of the prediction, the docked pose was ranked by using *i*GEMDOCK scoring function. From this virtual screening process, we finally got 13 chemical compounds that bound at different binding pockets of the selected proteins. The top ranked potential leads compounds from each selected protein molecules with their corresponding energy values are listed in Table I. Lesser the energy greater the acceptability of chemical as a drug. Molecules that scored best by *i*GEMDOCK scoring functions were identified as potential leads for tuberculosis drug discovery process.

Table 1: Showing iGEMDOCK energy values.

	Compound ID	***Chemical Name***	***1DQY***		***1KPI***		***1TQ8***	
S. No.			$E_{GEMDOCK}$	E_{pharma}	$E_{GEMDOCK}$	E_{pharma}	$E_{GEMDOCK}$	E_{pharma}
1	ZINC03874317	Myricetin	-116.3	-159.4	-	-	-	-
2	ZINC00517261	Isorhamnetin	-	-	-	-	-94.3	-118.9
3	ZINC00119983	Cianidanol	-	-	-115	-165	-	-
4	ZINC003911	Fisetin	-	-	-	-	-104.1	-133
5	ZINC03869768	Kaempferol	-	-	-126.9	-148.4	-	-
6	ZINC00039092	Hesperetin	-111.5	-152.1	-	-	-96.4	-123.1
7	ZINC03869685	Quercetin	-	-	-127.8	-136.4	-94	-134.3
8	ZINC00391840	Pelargonidin chloride	-	-	-	-	-92	-132.4
9	ZINC18185774	Luteolin	-105.1	-163.6	-	-	-	-
10	ZINC03871576	Apigenin	-102.1	-154.3	-	-	-	-
11	ZINC00058117	Eriodictyol	-108.3	-144.8	-	-	-	-
12	ZINC00001785	Naringenin	-103.4	-151	-117.2	-156.7	-	-
13	ZINC00001219	Coumestrol	-	-	-124.6	-158.8	-	-

Pharmacological Interactions

The pharmacological interactions derived by *i*GEMDOCK are useful for identifying lead compounds and understanding ligand binding mechanisms for a therapeutic target. In *i*GEMDOCK, an interaction conservation is viewed as a pharmacological preferences and an interaction is considered as the pharmacological interaction if W_j >=0.4 [23]. Here, for the H and V profile of all the selected target shows that all the residues have pharmacological preferences >=0.4 (Table II). The residues that lie within 8 Å unit area of ligand that interact with it through their Side chain and Main chain were considered as active site residues.

While mining the pharmacological interactions of three selected target proteins, we observed that for 1KPI and 1DQY, among the 9 predicted pharmacological interactions, 9 of 9 residues agree with the hot spots. For 1TQ8, 8 of 8 residues agree with the hot spots (Table II).These results indicate that the pharmacological interactions from screening compounds are often essential for the ligand binding.

Table II: Pharmacological interactions and consensus interaction ratio.

PDB ID	*Predicted Pharmacological Interactions*	*Consensus interaction ratio**
1DQY	Asp 38-H-S (0.55)	0.80
	Arg 41-H-M (0.76)	0.90
	Ala 42-H-M (0.54)	0.80
	Asn 52-H-S (1.00)	1.00
	Trp 262-H-S (0.58)	0.80
	Leu 40-V-M (0.93)	1.00
	Arg 41-V-M (1.00)	1.00
	Arg 41-V-S (0.99)	1.00
	Trp 262-V-M (0.92)	1.00
1KPI	Tyr 24-H-S (1.00)	0.90
	Gly 82-H-M (0.93)	0.90
	Gln 107-H-S (0.61)	0.70
	Tyr 24-V-S (0.89)	0.90
	Gly 80-V-M (0.70)	0.80
	Leu 103-V-S (0.68)	0.80
	Ala 146-V-M(1.00)	1.00
	His 149-V-S (0.47)	0.70
	Phe 150-V-S(0.70)	0.80
1TQ8	Arg 32- H-M (1.00)	1.00
	Ile 82- H-M (0.49)	0.70
	Asp 85- H-S (0.51)	0.70
	Arg 89- H-S (0.70)	0.80
	Gly 26- V-M (0.97)	1.00
	Ser 27-V-M (0.68)	0.80
	Asp 28-V-M (0.71)	0.80
	Arg 89-V-S (1.00)	1.00

*The consensus interaction ratio of the residue *i* is defined as A_j/A, where A_j is the number of active compounds interacting to the residue *i* and A is the total number of active compounds. residue *i* is considered as "hot spot" if the consensus interaction ratio >=0.5. [23].

H and V are interaction types. M and S are Main chain and Side chain.

Post Screening Analysis

In the post screening analysis PDB ID- 1DQY shows that the compound ID ZINC03874317 (Myricetin), ZINC18185774 (Luteolin) and ZINC00039092 Hesperetin) have better drug activity (Figure 3). In 1KPI, the post screening analysis predicted that the compound ID ZINC03869768 (Kaempferol), ZINC03869685 (Quercetin), ZINC0119983 (Cianidanol) and ZINC00001219 (Coumestrol) have better drug activity with the target protein (Figure 4). Compound ID ZINC003911 (Fisetin), ZINC0039092 (Hesperetin) and ZINC03869685 (Quercetin) were observed as potential drug candidate with good docking activity with the target protein 1TQ8 (Figure 5). It is also observed that Quercetin has good binding activity against the target proteins, PDB ID- 1KPI and 1TQ8. In PDB ID- 1DQY and 1TQ8, Hesperetin have good binding activity. The interactions and fitness score of the compound suggest that these leads can be formulated as an anti tubercle drug.

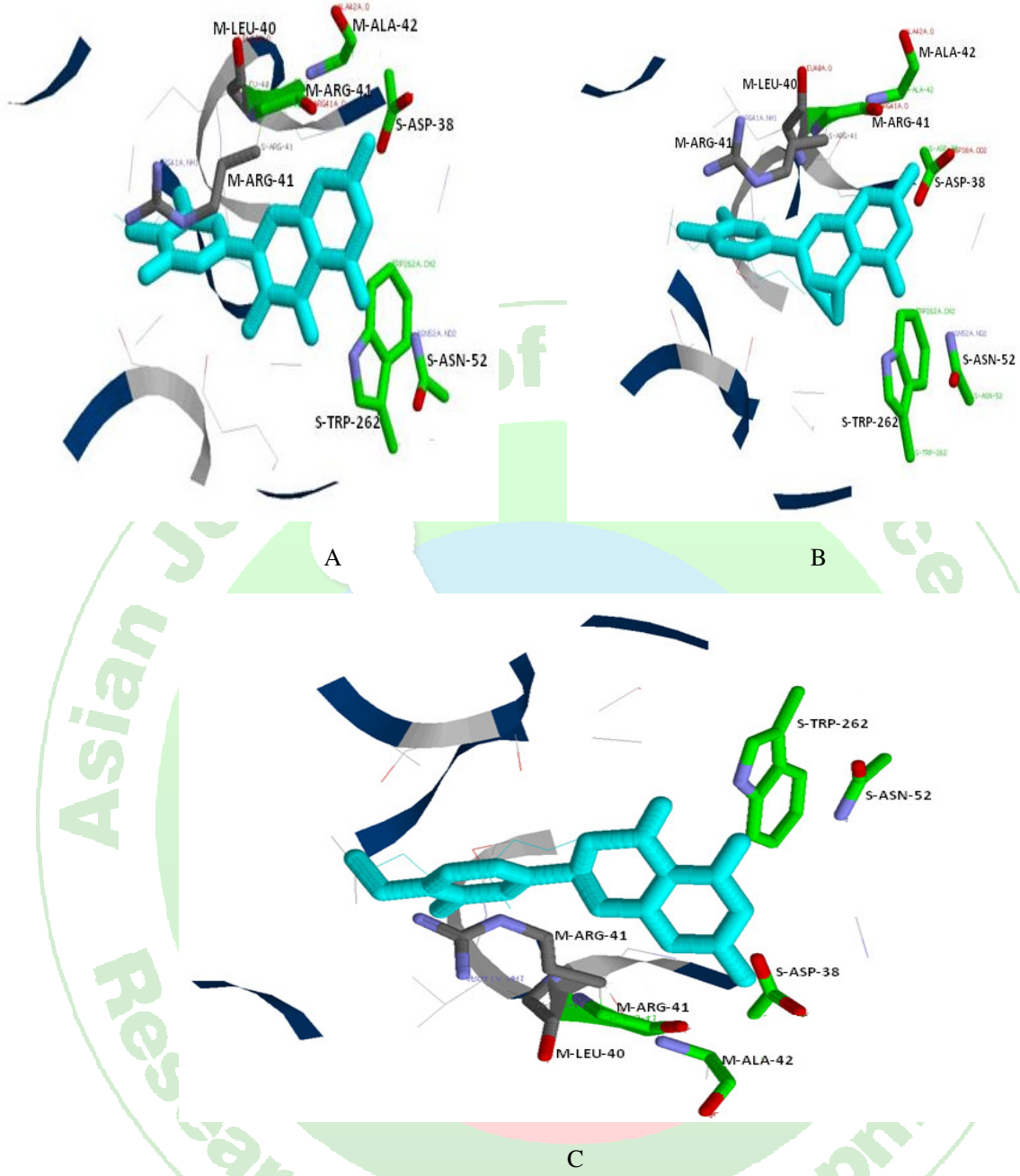


Figure 3: Predicted docking pose of (A) ZINC03874317 (B) ZINC18185774 (C) ZINC00039092 lie within the active site of the target protein, PDB ID- 1DQY. Cyan color represents the corresponding ligand molecule, green and grey color represents the amino acids involved in hydrogen bonding and van der Waals interactions respectively.

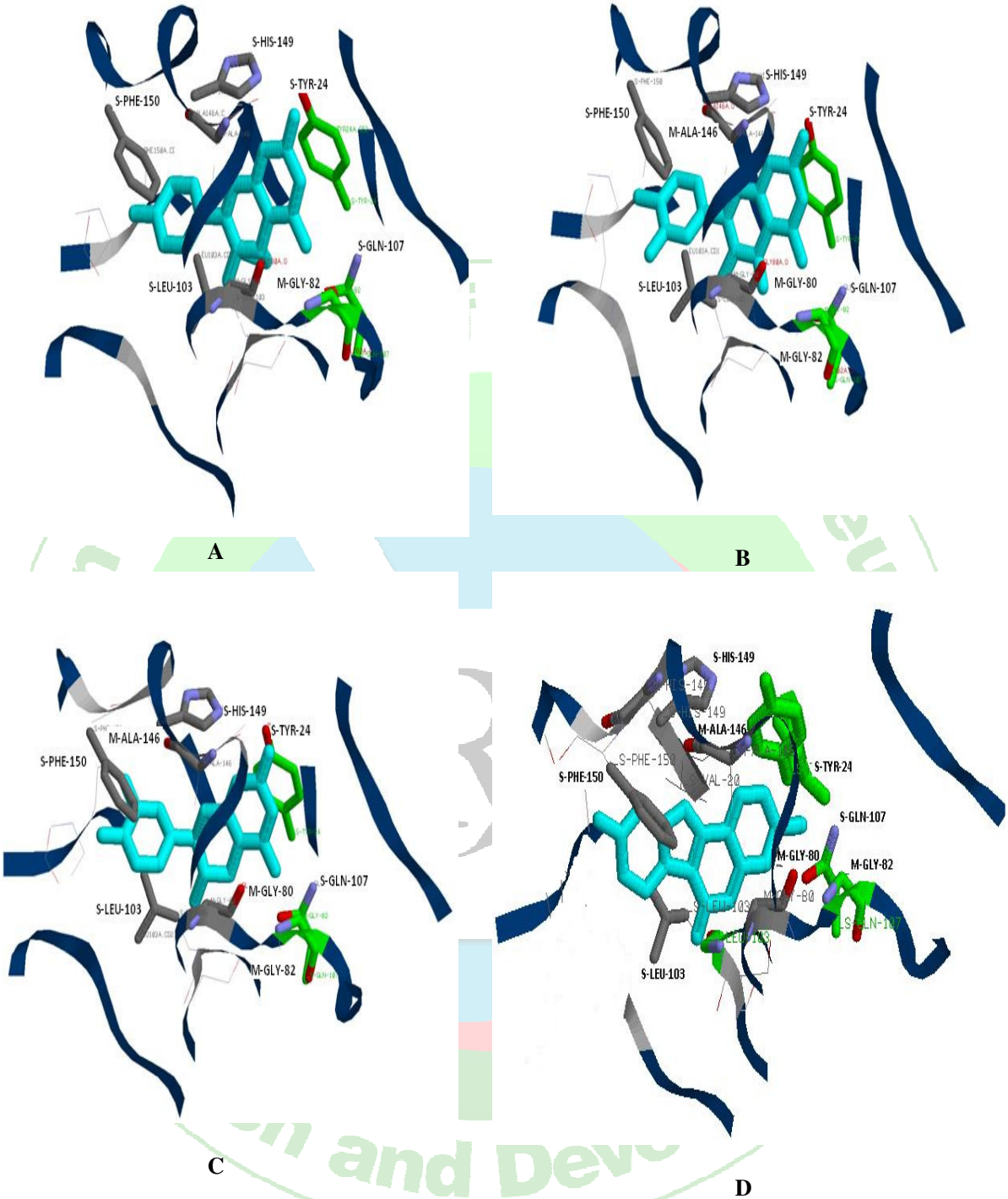


Figure 4: Predicted docking pose of (A) ZINC03869768 (B) ZINC03869685 (C) ZINC0119983 and (D) ZINC00001219 lie within the active site of the target protein, PDB ID- 1KPI. Cyan color represents the corresponding ligand molecule, green and grey color represents the amino acids involved in hydrogen bonding and van der Waals interactions respectively.

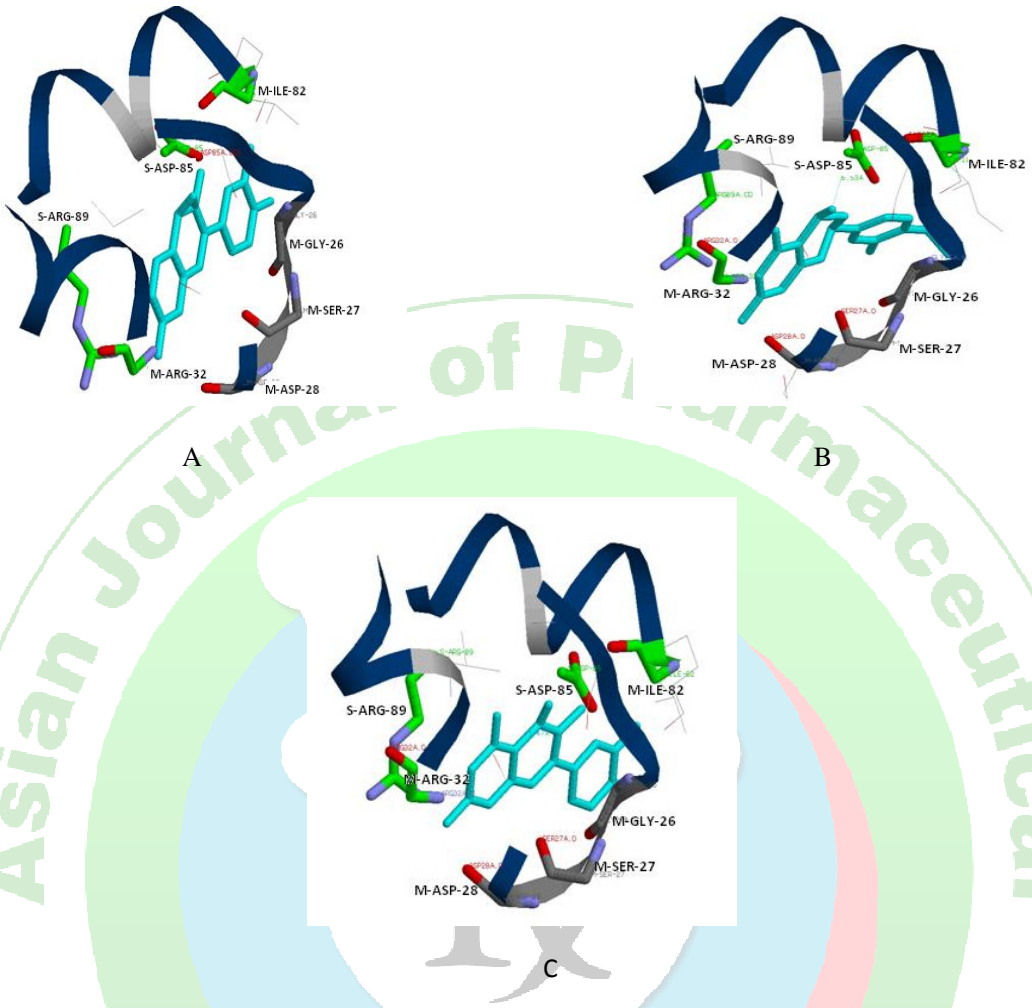


Figure 5: Predicted docking pose of (A) ZINC003911 (B) ZINC0039092 (C) ZINC03869685 lie within the active site of the target protein, PDB ID- 1TQ8. Cyan color represents the corresponding ligand molecule, green and grey color represents the amino acids involved in hydrogen bonding and van der Waals interactions respectively.

Satisfying Lipinski's rule of five?

No discussion of drug-likeness would be complete without reference to the influential Rule of 5 (Ro5) which is essentially a statement of property distributions for compounds taken into Phase II clinical trials. Lipinski's Rule of Five is a rule of thumb to evaluate drug likeness, or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most medication drugs are relatively small and lipophilic molecules [24].

The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their **A**bsorption, **D**istribution, **M**etabolism, and **E**xcretion ("ADME"). The rule is important for drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity, as well as drug-like properties as described by Lipinski's rule [25]. The 13 high ranked lead molecules were prioritized to follow Lipinski's rule-of-five based on the drug likeliness properties is listed in Table III.

Table III: Molecular properties including Lipinski's rule of five and drug likeness.*

Compound ID	*Chemical name*	*Molecular Formula*	*M.W[a] g/mol*	*X logP[b]*	*TPSA[c]*	*Hbond donor*	*Hbond acceptor*	*No. of r b[d]*
ZINC03874317	Myricetin	$C_{15}H_{10}O_8$	318.24	-1.39	152	5	8	1
ZINC00517261	Isorhamnetin	$C_{16}H_{12}O_7$	316.26	1.99	120	4	7	2
ZINC00119983	Cianidanol	$C_{15}H_{14}O_6$	290.27	1.37	110	5	6	1
ZINC003911	Fisetin	$C_{15}H_{10}O_6$	286.23	1.97	111	4	6	1
ZINC03869768	Kaempferol	$C_{15}H_{10}O_6$	286.23	2.17	111	4	6	1
ZINC00039092	Hesperetin	$C_{16}H_{14}O_6$	302.27	-1.94	96	3	6	2
ZINC03869685	Quercetin	$C_{15}H_{10}O_7$	302.23	1.68	131	5	7	1
ZINC00391840	Pelargonidin chloride	$C_{15}H_{11}O_5Cl$	306.69	-0.26	92	4	5	1
ZINC18185774	Luteolin	$C_{15}H_{10}O_6$	286.24	1.97	111	4	6	1
ZINC03871576	Apigenin	$C_{15}H_{10}O_5$	270.24	2.46	91	3	5	1
ZINC00058117	Eriodictyol	$C_{15}H_{12}O_6$	288.25	1.63	107	4	6	1
ZINC00001785	Naringenin	$C_{15}H_{12}O_5$	272.25	2.12	87	3	5	1
ZINC0001219	Coumesterol	$C_{15}H_8O_5$	268.22	2.54	84	2	5	0

[a]Molecular Weight [b]Octanol/Water partition coefficient [c]Topological Polar Surface Area

[d]No. of rotatable bonds.

*Data collected from Zinc database and PubChem [26].

CONCLUSION

We found *in silico* drug docking a better approach to check utility of any chemical as a drug before going through any *in vivo* or *in vitro* analysis to shorten out the experiments and cost cutting. Our study suggest that the flavonoid compounds, Coumestrol, Fisetin, Hesperetin, Myricetin, and Quercetin can be used as a lead molecule against the cell wall proteins of *M. tuberculosis* for performing *in vitro* and *in vivo* study. The set of molecules identified by us in this study are very likely to serve as potential leads in the search for new drugs against tuberculosis.

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