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De-Novo Drug Design of Shorter Chain Peptide as Antitubercular Agents

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ABSTRACT

Tuberculosis remains a deadly disease throughout worldwide and the emergence of Multidrug resistance makes us an urgent need to find a therapeutic lead using bio-friendly shorter chain peptides. 200 peptide templates (di, tri,tetra & penta peptide 50 each) were selected for rational drug design approach viz., Lipinski filters, Boman index, molinspiration & Swiss dock procedure in which 1 amino acid was mandatory chosen from past literature survey & the rest of combinations by trite and trial basis. Among the results obtained from Lipinski only 13 peptides, namely (Try-Asp, Ile-Arg, Leu-Arg, Glu-Arg, Asp-Glu, Asp-Pro-Phe, Gly-Ala-Asp, Met-Asp-Val, Gly-Ala-Leu-Asp, Pro-Gly-Asp-Ala, Gly-Ala-Leu-Arg-Ser, & Ala-Cys-Gly-Ser-Asp are predicted to be best outcome leads against tuberculosis. Further among 13 leads subjected to Boman index calculations & molecular properties of molinspiration, we found that 9 leads were considered as better targets (rejected leads : Pro-Gly-Asp-Ala, Gly-Ala-Asp, Gly-Ala-Leu-Arg-Ser, Ala-Cys-Gly-Ser-Asp for both HGPRT enzyme and TLR-2 receptor. Among the 9 leads subjected to docking against Hypoxanthine Guanine Phospho Ribosyl Transferase enzyme & Toll like receptor 2, we found that dipeptide Glu-Arg (D-E) is considered to be the most potent therapeutic lead against HGPRT enzyme with its full fitness energy as -395546 k.cal/mols and the dipeptide Asp-Glu (D-E) was found to be the most potent therapeutic lead against TLR-2 receptor for TB with its full fitness energy as -978870 k.ca/mol respectively. Further these dipeptide lead can be explored to test preclinical efficacy & comparison of therapeutic potency will be validated through structure based drug design in near future.

Key words: Lipinski rule (ROS), Antimicrobial peptide(Boman Index), Moleinspiration & Swiss docking.

INTRODUCTION

Tuberculosis (TB) poses a major worldwide public health problem[1]. The increasing prevalence of TB, the emergence of multi-drug-resistant strains of *Mycobacterium tuberculosis* and the devastating effect of co-infection with HIV have highlighted the urgent need for new strategies and tools to control the disease. The available TB vaccine, the bacillus Calmette Guerin (BCG), is an attenuated strain of the closely related organism *Mycobacterium bovis*. Although widely used, its efficacy has been very variable in clinical trials conducted in different parts of the world (Fine, 2001). A new vaccine and new drugs are urgently needed to combat this devastating disease. [2]

Rational drug designing is the discovery of the lead compound in the most difficult step

Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) is an enzyme encoded in humans by the *HPRT1* gene. HGPRT is a transferase that catalyzes conversion of hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate. This reaction transfers the 5-phosphoribosyl group from 5-phosphoribosyl 1-pyrophosphate (PRPP) to the purine. HGPRT plays a central role in the generation of purine nucleotides through the purine salvage pathway. [3]

Toll-like receptor 2 also known as TLR2 is a protein that in humans is encoded by the *TLR2* gene. TLR2 has also been designated as CD282 (cluster of differentiation 282). TLR2 is one of the toll-like receptors and plays a role in the immune system. TLR2 is a membrane protein, a receptor, which is expressed on the surface of certain cells and recognizes foreign substances and passes on appropriate signals to the cells of the immune system [4]

TLRs mediate cellular activation by components of mycobacteria and cooperate with other branches of the innate immune system to effectively destroy mycobacteria. Two critical effectors' functions of the innate immune system are phagocytosis and the activation of direct anti-microbial pathways.

Pathphysiology Droplet nuclei with bacilli are inhaled, enter the lung, And deposit in alveoli. Macrophages and T lymphocytes act together to try to contain the infection by forming granulomas. In weaker immune systems, the wall loses integrity and the bacilli are able to escape and spread to other alveoli or other organs.[5]

MATERIALS AND METHODS

2.1. MATERIALS;

Computer configuration is performed in the HP computer having Processor: - Intel ® core (TM) i3 CPU M370 @2.40GHz 2.40z. the Installed memory of the computer is (RAM):- 2.00 GB (1.86 GB usable). Type of the system is 32-bit operating system, x-64 based processor and no pen or touch input is available for this display

2.2. METHODOLOGY:-

1. Rational drug design:-

The discovery of the lead compound in the most difficult step. The discovery of phrmacophore follows, after a series of serial operation on the lead molecules. Optimization involves further structural manipulations on the pharmacophore, which is conjunction with physical organic measurement and biological testing **(**Nagarajan K *et al.,* 2016). Lead to finding of the best compound for particular purpose. Often (quantitative) structure activity relationship also included.

Of course, the discovery of a new medicine is still far away from this stage; however, with the addition of studies on drug metabolism, and after the study of the molecular mechanism of action, the main work of the medicinal chemist in the discovery of new therapeutic agent is completed. Number of approaches in rational drug design, the molecular mechanism of drug action, Drug metabolizing enzyme action upon the structure of to drug molecule, Patho-biochemistry and path physiology of the target disease.

In the development of drug starting from compounds stored in data banks. Structures, physicochemical properties, and biological activities are taken, a few are selected, through mentioned strategies are applied. Totally 200 combinations of dipeptides, tripeptide, tetrapeptide, pentapeptide has been subjected to Lipinski rule. The selection of amino acid in dipeptide is based on past literatures with 1 amino acid as mandatory in combination with rest of amino acid by hot & trial approaches[6]

2. **Lipinski rule;** Lipinski's rule of five also known as the Pfeiffer's rule of five or simply the Rule of five (RO5) 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 orally administered drugs are relatively small and moderately lipophilic molecules.

The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). However, the rule does not predict if a compound is pharmacologically active.

The rule is important to keep in mind during drug discovery when a pharmacologically active lead structure is optimized step-wise to increase the activity and selectivity of the compound as well as to ensure drug-like physicochemical properties are maintained as described by Lipinski's rule. Candidate drugs that conform to the RO5 tend to have lower attrition rates during clinical trials and hence have an increased chance of reaching the market [7].
Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria: No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds), No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms), A molecular mass less than 500 daltons, An octanol-water partition coefficient log P not greater than 5. During drug discovery, lipophilicity and molecular weight are often increased in order to improve the affinity and selectivity of the drug candidate. Hence it is often difficult to maintain drug-likeness (i.e., RO5 compliance) during hit and lead optimization. Hence it has been proposed that members of screening libraries from which hits are discovered should be biased toward lower molecular weight and lipophilicity so that medicinal chemists will have an easier time in delivering optimized drug development candidates that are also drug-like. Hence the rule of five has been extended to the rule of three (RO3) for defining lead-like compounds.

3. Antimicrobial peptide database

This comprehensive database for antimicrobial peptides is manually curated based on a set of data-collection criteria. There are 113 human host defense peptides, 1017 active peptides from amphibians, 100 fish peptides, 22 reptile peptides, 38 from birds, 465 from arthropods, 136 from chelicerata, 45 from crustaceans, 7 from myriapods, 273 from insects, 43 from spiders, 65 from scorpions, 35 from molluscs; and more. A universal bacterial peptide nomenclature;

A unified peptide classification, Total Hydrophobic ration, Total Net charge, Total Pro(P) ration, Total Trp (W) ration, Total Gly (G) ration (http://aps.unmc.edu/AP/design/design_main.php).

BOMAN INDEX

Defined by this database in 2003 in mamory of hans bowman who called it protein-binding-potential. Bowman index is the sum of the free energies of the respective side chains for transfer from cyclohexane to water taken from Radzeka and wolfenden and divided by the total number of the residues of an antimicrobial peptide. The calculated value are negative (except for te hybride peptide) but the + and – are reversed (by net).

The boman index estimates the potential for a protein to bind to other proteins[8] (Boman et al., 2003). In other words, a high boman index value indicates that the designed lead will be multifunctional or play a variety of different roles within the cell due to its ability to interact with a wide range of proteins

Molinspration

Molinspiration offers broad range of cheminformatics software tools supporting molecule manipulation and processing, including SMILES and SD file conversion, normalization of molecules, generation of tautomers, molecule fragmentation, calculation of various molecular properties needed in QSAR, molecular modeling and drug design, high quality molecule depiction, molecular database tools supporting substructure and similarity searches. Our products support also fragment-based virtual screening, bioactivity prediction and data visualization. Molinspiration tools are written in Java, therefore can be used practically on any computer platform. Molinspiration supports internet chemistry community by offering free on-line services for calculation of important molecular properties (logP, polar surface area, number of hydrogen bond donors and acceptors and others), as well as prediction of bioactivity score for the most important drug targets (GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors).[9]

4. **Swiss dock software and target protein for docking**

Swiss Dock was used for docking of selected lead compounds via enzyme HGPRT & receptor TLR-2. Docking studies helps in prediction of the preferred orientation of a ligand with the binding site on a protein. Molecular docking was used to determine appropriate binding orientations and conformations of various chemical compounds at the target site. After docking, all the legend confirmations were ranked on the basis of their binding energy.

RESULTS AND DISCUSSION

3.1 Lipinski Rule study

Five parameters for 250 peptide molecules were determined using the software online on the website of IIT Delhi bio-informatics. The five parameters are:-

- Molecular weight
- Number of hydrogen donor group
- Number of hydrogen acceptor group
- Log P value
- Molar refractivity

Table 1. Lipinski rules of drug design for dipeptide

S.No	Molecule	Mol.Wt	H. Bond donar	H. Bond Acceptor	Log P	Molar Refactivity
1	Y-R	0	0	0	0	0
2	Y-D	0	0	0	0	0
3	W-R	0	0	0	0	0
4	W-D	314	2	8	0.72304	80.511391
5	V-R	0	0	0	0	0
6	V-D	228	3	7	2.666031	50.983093
7	T-R	226	6	9	1.71666031	63.509296
8	T-D	228	4	8	3.39443	47.843895
9	S-R	256	7	9	2.869361	63.176998
10	S-D	216	4	8	4.47383	43.160896
11	R-Y	291	6	9	0.415921	77.192192
12	R-W	355	5	9	0.63831	98.636497
13	R-R	321	9	11	2.341191	85.637299
14	P-R	262	5	7	0.89626	68.459999
15	R-L	278	6	8	0.75079	73.499191
16	R-G	226	6	8	2.231861	57.148197
17	R-D	280	5	10	1.71519	66.323502
18	P-R	264	7	7	1.59713	67.6474
19	P-D	224	3	6	2.844001	48.4646
20	M-R	257	3	7	2.427641	58.163097
21	L-R	239	2	7	0.26946	56.208393
22	M-D	257	3	7	2.427641	58.163097
23	L-D	240	3	7	2.499931	55.506096
24	K-D	252	4	8	1.20706	59.244797
25	I-R	237	2	7	0.49346	56.114395
26	I-D	237	2	7	0.10007	55.036896
27	H-R	306	7	7	1.89586	78.382896
28	H-D	266	5	5	3.763	59.100498
29	G-D	186	3	3	2.79963	37.955898
30	F-D	275	3	3	0.25563	67.657097
31	E-R	256	2	9	2.70556	51.018394
32	E-D	256	2	9	2.70556	51.018394
33	D-R	283	5	10	3.05329	63.546494
34	D-K	253	3	8	1.796391	57.270092
35	D-I	240	4	7	3.067201	55.432796
36	D-E	258	2	9	2.48156	51.112389
37	D-D	343	3	9	5.32613	45.699097
38	C-R	0	0	0	0	0
39	C-D	232	4	7	3.756001	49.604749
40	A-R	240	6	8	1.905131	60.016998
41	A-D	200	3	7	3.44623	41.749096
42	R-R	321	9	11	2.341191	85.637299
43	P-R	262	5	7	0.89626	68.459999
44	R-L	278	6	8	0.75079	73.499191
45	R-G	226	6	8	2.231861	57.148197
46	R-D	280	5	10	1.71519	66.323502
47	H-R	306	7	7	1.89586	78.382896
48	H-D	266	5	5	3.763	59.100498
49	G-D	186	3	3	2.79963	37.955898
50	F-D	275	3	3	0.25563	67.657097

Table 2: Lipinski rule of Tripeptide

S.No	Molecules	Mol. Wt	H. Bond Donar	H. Bond Acceptor	Log P	Molar refractivity
1	A-D-V	299	5	9	3.00833	70.374496
2	A-I-D	312	5	9	2.61823	74.991493
3	A-I-R	351	9	10	1.59536	94.080292
4	A-R-V	339	9	10	1.761461	89.557289
5	C-D-A	301	5	9	3.80873	69.121498
6	C-D-M	0	0	0	0	0
7	C-R-M	400	10	10	1.30927	105.895607
8	D-D-F	390	5	11	4.688	88.776497
9	D-D-R	394	6	14	3.370119	89.738205
10	D-P-F	373	4	9	1.17872	92.620796
11	D-R-E	411	8	14	5.186558	91.42659
12	D-R-F	430	10	12	4.00908	107.885986
13	D-W-F	0	0	0	0	0
14	E-D-M	386	4	11	2.15287	87.167793
15	E-R-D	411		14	5.553759	93.673599
16	F-R-K	440	9	11	0.8529	122.495308
17	F-R-P	415	7	10	0.99394	112.598686
18	G-A-D	257	5	9	4.178629	56.523499
19	G-D-A	272	4	10	5.060359	55.539799
20	G-R-D	340	7	12	3.785689	78.3209
21	I-D-C	341	5	9	2.86243	82.878494
22	I-R-C	383	8	10	1.03396	102.988594
23	K-D-M	382	5	10	0.0871	95.467499
24	K-D-S	337	6	11	4.287159	77.391701
25	K-R-M	423	9	11	0.428871	112.783096
26	K-R-S	377	11	12	3.607559	96.501198
27	M-D-S	341	5	10	3.64454	76.939003
28	M-D-V	357	4	9	0.11713	87.834785
29	M-R-S	381	9	11	2.59767	96.121796
30	M-R-V	398	8	10	0.508871	107.14859
31	P-R-Y	427	9	10	1.28209	112.719879
32	R-D-A	354	8	12	4.063959	85.106598
33	R-D-K	405	9	13	3.711989	101.89311
34	R-G-D	342	9	12	4.797328	80.5103
35	S-C-R	359	9	11	2.95416	90.497391
36	S-D-L	327	8	10	3.869829	76.309296
37	V-A-R	337	8	10	1.62786	90.296593
38	S-L-R	367	5	11	1.922091	96.304695
39	V-D-D	340	5	11	5.112229	74.230499
40	V-D-I	337	5	9	2.28603	84.037491
41	V-D-M	356	9	9	2.33734	87.038491
42	V-R-D	380	8	12	3.86538	93.413292
43	V-R-I	377	6	10	0.68156	104.053596
44	W-D-Y	478	8	11	1.54999	124.048264
45	W-R-A	426	5	11	0.245261	119.216599
46	Y-D-C	394	7	10	0.37216	95.908592
47	Y-D-W	480	10	11	1.83009	123.293961
48	Y-R-C	435	6	11	3.86538	93.413292
49	D-R-V	445	17	21	1.922091	96.304695
50	W-E-R	678	14	12	3.86538	93.413292

Table 3:- Lipinski rules of Tetrapeptide

S.No	Molecules	Mol.wt	H.Bond Donar	H.Bond Acceptor	Log P	Molar refractivity
1	A-I-C-D	414	7	11	2.980729	102.363899
2	A-M-S-R	457	11	12	2.658969	118.717796
3	A-V-R-M	467	10	12	0.851169	126.540001
4	C-D-M-W	544	6	12	1.330121	138.009232
5	C-M-D-I	476	9	10	1.602551	121.718399
6	C-M-R-A	0	0	0	0	0
7	C-M-R-D	516	10	14	3.307468	129.331009
8	C-M-T-R	499	11	3	1.599769	13.381836
9	C-T-D-M	460	6	12	3.747668	108.904205
10	D-A-P-F	448	7	11	2.146889	113.545876
11	D-E-M-C	490	7	13	4.120538	113.535889
12	D-E-R-T	511	9	17	6.387688	116.698303
13	D-E-S-T	440	7	15	8.04672	90.88739
14	D-G-C-M	421	7	11	3.198139	100.88739
15	C-R-F-K	541	11	13	0.9985	149.081848
16	D-M-S-R	500	10	15	3.971597	122.7411505
17	D-R-H-T	518	9	17	4.711518	126.764313
18	D-R-W-F	616	10	15	2.053209	164.180206
19	D-S-M-C	414	7	12	4.310438	96.6744
20	E-D-M-C	490	6	12	5.500838	111.673195
21	F-D-H-K	339	10	14	2.832228	140.54509
22	F-H-R-L	564	11	14	0.514159	157.430923
23	F-H-T-R	552	12	15	1.651718	148.982666
24	F-P-D-Y	540	8	12	1.60409	139.179749
25	F-W-Y-D	628	9	13	2.213269	166.72049
26	F-Y-R-W	666	10	14	0.08938	185.781235
27	G-A-L-D	367	6	11	1.344161	90.374191
28	G-A-V-R	396	11	12	2.270968	103.391487
29	H-K-P-R	525	12	14	1.295389	142.024002
30	H-K-R-D	544	12	16	3.884387	137.982498
31	H-R-F-W	641	12	14	0.26608	177.675613
32	H-T-D-F	511	7	14	4.023618	126.410896
33	H-T-D-P	511	7	11	4.023618	126.410896
34	I-C-D-M	471	7	11	2.30974	119.027893
35	K-G-C-D	415	8	12	3.517158	100.9506
36	K-P-W-R	580	11	14	0.99261	164.802963
37	K-R-D-T	508	12	16	3.257788	126.883308
38	K-R-F-D	552	11	15	3.074549	144.956818
39	K-W-R-A	550	11	14	0.776251	154.115891
40	L-S-M-R	0	0	0	0	0
41	M-A-R-G	427	11	12	2.241138	112.3657
42	M-S-R-L	496	9	13	1.566799	130.079315
43	P-D-Y-W	575	8	12	1.841221	148.945312
45	P-G-D-A	364	7	10	3.694699	82.724388
46	P-R-W-D	564	10	14	2.43072	147.20961
47	R-C-A-V	440	10	12	1.712659	117.616997
48	R-K-H-D	543	12	17	1.89143	138.947281
49	R-L-A-G	408	10	12	1.970159	109.68796
50	T-M-R-G	457	11	13	1.745889	118.877792

Table 4; Lipinski rules of Pentapeptide

S.No	Molecules	Mol.Wt	H.Bond Donar	H.bond Acceptor	Log P	Molar refractivity
1	A-C-G-S-D	442	8	14	4.964757	103.556602
2	A-G-V-L-D	469	9	13	2.912728	118.391289
3	A-G-V-L-R	509	12	14	1.308258	138.407471
4	A-R-G-I-T	506	9	15	2.338429	130.407318
5	C-A-L-D-V	513	8	13	2.04896	127.812607
6	C-A-L-V-R	551	12	14	1.162358	150.911575
7	C-A-V-L-R	564	13	14	3.525557	147.200745
8	C-D-R-PM	617	12	16	2.993169	158.300613
9	C-P-D-W-Y	782	8	14	2.37347	189.850739
10	C-R-D-P-Y	639	9	16	1.36913	159.03006
11	C-S-P-F-D	587	9	15	4.017417	140.950485
12	C-S-T-P-R	554	13	16	3.832568	139.526688
13	C-V-L-D-S	524	9	14	3.841427	130.279831
14	C-W-F-K-D	691	11	15	1.882747	185.272919
15	C-D-R-P-M	617	12	16	2.993169	158.300613
16	C-P-D-W-Y	728	8	14	2.372347	189.850739
17	C-R-D-P-Y	639	9	16	1.3693	159.03008
18	C-S-P-F-D	580	9	15	4.017417	140.950485
19	C-S-T-P-R	554	13	16	3.832568	139.526688
20	C-V-L-D-S	524	9	14	3.841427	130.279831
21	C-W-F-K-D	691	11	15	1.882747	185.272919
22	C-W-R-K-D	695	13	18	1.460879	184.855347
23	D-C-M-P-F	610	9	13	1.390398	157.770477
24	D-W-H-Y-W	805	12	18	1.912608	216.291733
25	E-S-M-P-D	607	9	17	5.540716	141.682465
26	E-S-M-P-R	612	13	17	3.891468	153.143784
27	G-A-L-D-S	453	7	14	2.36819	108.781906
28	G-A-L-R-S	492	12	15	3.387058	128.463913
29	M-A-V-D-C	530	9	13	2.416839	133.500336
30	M-A-V-D-R	581	12	16	3.397368	149.787552
31	P-F-D-M-E	631	9	14	3.261967	155.743988
32	P-F-D-M-R	655	12	15	2.271597	171.049164
33	R-C-M-P-E	630	10	16	1.447288	160.62973
34	R-E-D-P-K	638	13	19	4.434187	158.745316
35	R-E-R-P-Y	714	17	20	4.7814147	182.763199
36	R-H-I-K-D	653	14	19	3.607187	173.073059
37	R-H-I-K-M	671	14	17	0.608298	185.975113
38	R-S-C-M-A	0	0	0	0	0
39	R-W-H-T-I	706	15	18	0.972588	195.260864
40	S-C-D-M-I	0	0	0	0	0
41	S-C-R-M-I	600	12	15	0.954610	159.340724
42	S-G-V-D-M	520	10	15	5.317638	122.804085
43	S-G-V-R-M	544	14	15	2.744767	141.987000
44	S-K-S-D-P	525	10	16	5.140157	125.127502
45	S-R-K-S-P	567	13	17	3.197817	146.562332
46	T-D-E-S-C	545	10	17	6.504229	121.992607
47	T-D-R-K-E	636	13	20	5.386587	155.512115
48	T-D-V-K-R	605	15	18	3.698188	155.251801
49	T-R-E-S-C	601	12	19	3.585718	143.102615
50	C-G-D-C-K	512	10	14	3.303257	129.482010

By following Lipinski rule of fine the 13 molecule are selected are :-
W-D (Try-Asp) , I-R (Ile-Arg), L-R (leu-Arg), E-R (Glu-Arg), D-E (Asp-Glu), D-P-F (Asp-pro-PHe), G-A-D (Gly-ala-Asp), M-D-V (Met-asp-Val), G-A-L-D Glu-ala-leu-Asp), P-G-D-A (Pro-gly-asp-Ala), G-A-L-R-S (Gly-ala-leu-arg-Ser), G-A-L-D-S(Gly-ala-leu-asp-Ser), A-C-G-S-D (Ala-cys-gly-ser-Asp).

3.2**: ANTIMICROBIAL PEPTIDE DATABASE**

The proceeding of selecting 13 molecules are improved by the antimicrobial database as shown in the table 5.

Table 5:- Boman index rules of peptides from antimicrobial peptide database

S.No	Molecule	Total hydrophobic ration	Total Net Charge	Total Proline ratio	Total tryptophan ratio	Total glycine ratio	Bowman index
1	IR	50%	1	0%	0%	0%	5 Kcal/mol
2	LR	50%	1	0%	0%	0%	5 kcal/mol
3	WD	50%	1	0%	50%	0%	3.19 kcal/mol
4	DE	0%	0	0%	0%	0%	7.76 kcal/mol
5	DPF	33%	1	0%	0%	0%	1.91 kcal/mol
6	MDV	66%	1	0%	0%	0%	0.77 kcal/mol
7	GALD	50%	1	0%	0%	25%	0.26 kcal/mol
8	GALRS	40%	1	0%	0%	20%	1.61 kcal/mol
9	GALDS	40%	1	0%	0%	20%	0.89 kcal/mol
10	ACGSD	40%	1	0%	0%	20%	1.61 kcal/mol
11	ER	0%	0	0%	0%	0%	10.8kcal/mol
12	GAD	33%	1	0%	0%	33%	1.99 kcal/mol
13	PGDA	25%	1	25%	0%	25%	1.49 kcal/mol

3.3 MOLEINSPIRATION:-

Calculation of molecular properties and prediction of bioactivity of selected molecules as shown in table 6.

Table 6 :- Major drug target for the subjected lead compounds by molinspiration

S.No	Molecules	MiLog P	TPSA	N atom	Mol. Wt	GPCR ligand	Nuclear receptor	Protease inhibitor	Enzyme inhibitor
1	WD	2.47	145.51	23	319.32	0.77	0.12	0.83	0.52
2	IR	2.8	129.72	17	246.26	0.25	0.39	0.77	0.45
3	LR	2.77	129.72	17	246.26	0.38	0.11	0.84	0.47
4	ER	4.49	167.02	18	262.22	0.42	0.1	0.72	0.51
5	DE	4.49	167.02	18	262.22	0.42	0.1	0.72	0.51
6	DPF	2.88	150.03	27	377.4	0.71	0.2	1.05	0.44
7	GAD	4.44	158.82	18	261.23	0.34	0.35	0.66	0.34
8	MDV	3.41	158.82	24	363.44	0.36	0.09	0.88	0.44
9	GALD	3.8	187.91	26	374.39	0.49	0.06	0.85	0.34
10	PGDA	4.07	173.92	25	358.35	0.5	0.11	0.86	0.29
11	GALRS	4.9	261.84	35	502.57	0.56	0.28	0.92	0.38
12	GALDS	5.17	237.24	32	461.47	0.55	0.09	0.79	0.35

3.4 SWISS DOCKING FOR TARGET MOLECULES:-

Binding energy for each docking was calculated using a semi-empirical free energy force field. Out of these 9 docked molecules 4 are with the receptor TLR-2 (code 5D3I), was found to have the best affinity for the receptor and the 5 molecules are with the enzyme HGPRT (code 4RAN) are selected as shown in the table 7.

Table.7:- Docking results of peptide leads for antitubercular enzyme & receptor target

S.No	Molecule	Target	Protein code	full fitness kcal/mol	estimated ΔG kcal/mol
1	LR	Enzyme (HGPRT)	4RAN	-388753	1624.66
2	IR	Enzyme (HGPRT)	4RAN	-388799	1616.05
3	WD	Enzyme (HGPRT)	4RAN	-361307	1547.62
4	ER	Enzyme (HGPRT)	4RAN	-395546	1644.6
5	MDV	Enzyme (HGPRT)	4RAN	-362410	1630.31
6	DE	Receptor (TLR-2)	5D3I	-978870	1690.46
7	DPF	Receptor (TLR-2)	5D3I	-964514	1967.91
8	GALRS	Receptor (TLR-2)	5D3I	-879702	195.97
9	GALD	Receptor (TLR-2)	5D3I	-946231	1894.98

From the docking the selected molecules are having more full fitness which is to observed in below molecule estimated free energy

From the enzyme target (HGPRT), the molecules are; - Glu-Arg, Ile-Arg, Leu-Arg.
Having more antimicrobial activity as targeting enzyme. Fig 1, fig 2 & fig 3

Fig 1 Docking of dipeptide Glu-Arg,(E-R) with HGPRT enzyme as biological target

Fig 2 Docking of dipeptide (Ile-Arg),(I-R) with HGPRT enzyme as biological target

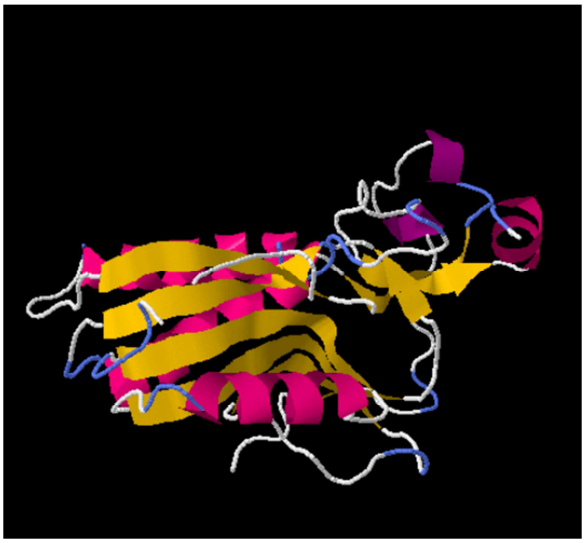


Fig 3 Docking of dipeptide Leu-Arg (Leu-Arg) with enzyme of HGPRT as biological target

From the docking the selected molecules are having more full fitness which is to observed in below molecule estimated free energy

From the receptor target (TLR-2), the molecules are; - Asp-Glu (D-E), Asp-Pro-Phe(D-P-F), Gly-Ala-leu-Asp(G-A-L-D)

Having more antimicrobial activity as targeting receptor Fig 4, Fig 5 & Fig 6.

Fig 4 Docking of dipeptide Asp-Glu(D-E) with the receptor TLR-2 as biological target

Fig 5 Docking of tripeptide Glu-Pro-Phe(D-P-F) with receptor TLR-2 as biological target

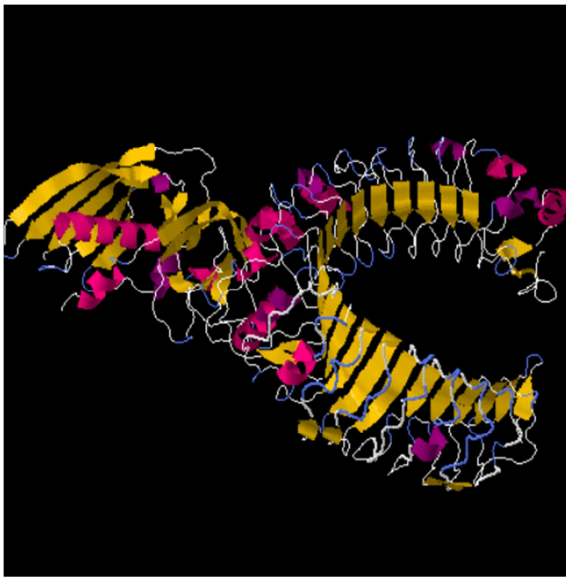
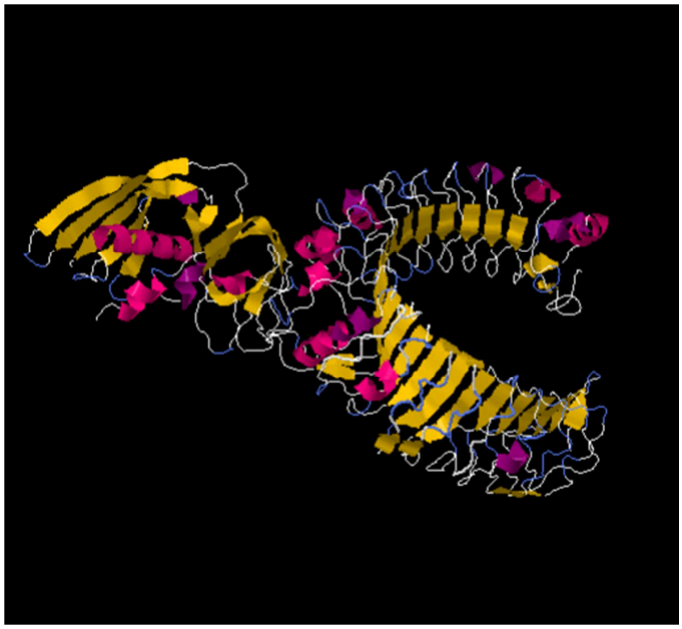


Fig 6 Docking of tetrapeptide Glu-Ala-Leu-Asp(G-A-L-D) with receptor TLR-2 as biological target



CONCLUSION

In this study, we conclude that the amino acid or peptide molecule namely D-E (Asp-Glu) , I-R (Ile-Arg), L-R (leu-Arg), E-R (Glu-Arg), D-P-F (Asp-pro-Phe),, G-A-L-D Glu-ala-leu-Asp), was found to be most potent antimicrobial compound where as the swiss dock model used to determine and confirm appropriate binding orientations and conformations at binding site with protein shows that dipeptide Glu-Arg (D-E) is considered to be the most potent therapeutic lead against HGPRT enzyme with its full fitness energy as -395546 k.cal/mol and the dipeptide Asp-Arg (E-R) was found to be the most potent therapeutic lead against TLR-2 receptor for TB with its full fitness energy as -978870 k.ca/mol respectively. Further these dipeptide leads can be explored to test preclinical efficacy & comparison of therapeutic potency will be validated structure based drug design in near future through enzyme & receptor to have the best affinity for the molecule.

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