Computational Identification of Phytoconstituent Leads for Leishmaniasis: Bridging Molecular Docking and Nanostructured Lipid Carrier (NLC) Engineering

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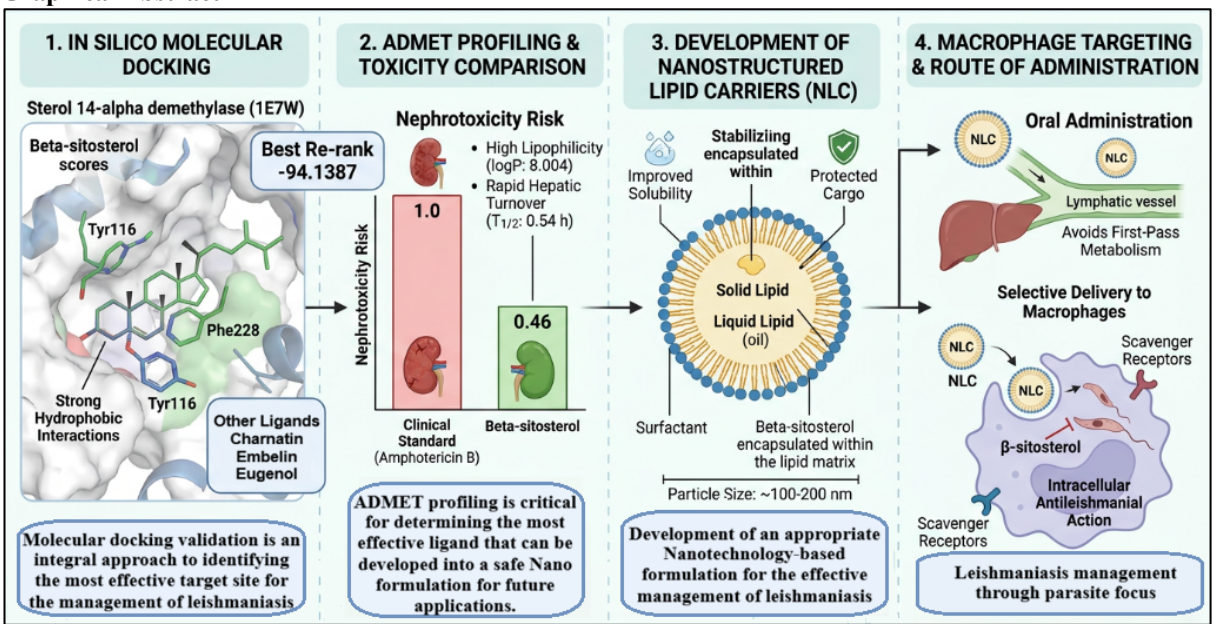
Graphical Abstract



Molecular docking of several receptors (PDB: 1E7W, 6MJO, 7JUB, 4O2Z, 6WBG, 1E92, and 1E7W) showed good hydrophobic interaction with Tyr116 and Phe228 residues to give the best re-ranking score of -94.1387 among all natural ligands. The ADMET analysis found that although it is highly lipophilic (log P = 8.004) and undergoes rapid hepatic degradation (T1/2 = 0.54 h), Beta-sitosterol is less nephrotoxic (0.46) than Amphotericin B (1.0). To address the limitations associated with its biopharmaceutical behaviour, the compound was loaded into a nanostructured lipid carrier (NLC;100-200nm size) made up of solid and liquid lipids along with stabilizers. These carriers, on oral administration, are transported to the intestinal lymphatic system to evade the first-pass effect.

Abstract

Background: Visceral leishmaniasis continues to pose a major global health challenge. The current gold-standard treatments, such as Amphotericin B, are limited due to their significant systemic toxicity, particularly nephrotoxicity, and their poor oral bioavailability. However, natural phytoconstituents present a promising opportunity for discovering safer, orally effective antileishmanial agents.

Objective: This study evaluated the antileishmanial potential and pharmacokinetic profiles of Beta-sitosterol, Charantin, Embelin, and Eugenol compared to a clinical standard to support a specialized nanoformulation strategy.

Methods: *In silico* molecular docking was conducted across 7 leishmanial and host receptors (1AI9, 6MJO, 7JUB, 4O2Z, 6WBG, 1E92, and 1E7W). Comprehensive ADMET profiling was carried out using ADMETlab 3.0 to assess drug-likeness, metabolic stability, and organ-specific toxicological risks.

Results: Beta-sitosterol has emerged as a promising multi-target compound, achieving a Re-rank score of -94.1387 against Sterol 14-alpha demethylase (1E7W). Significant hydrophobic interactions with Tyr116 and Phe228 strengthen binding interactions. Toxicological assessments indicate a lower nephrotoxicity risk for Beta-sitosterol (0.46) compared to the clinical standard (1.0). However, it is highly lipophilic (log P: 8.004) and has a rapid hepatic turnover (T1/2: 0.54 h), posing metabolic challenges.

Conclusion: Beta-sitosterol represents an efficient and non-toxic agent for leishmaniasis treatment. Solubility and metabolic issues necessitate the creation of Nanostructured Lipid Carriers (NLC). The use of NLC will increase lymphatic distribution, bypassing the first pass effect, thus facilitating selective delivery of the agent to macrophages. This approach integrates both high receptor affinity and physiological considerations for successful therapy.

Keywords: Leishmaniasis, *In-silico* study, Targeted Drug Delivery System (TDDS) ADMET Study, Phytoconstituents, Nanostructured Lipid Carriers (NLCs)**.**

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1. Introduction

Leishmania is a genus of protozoan parasites that are obligate intracellular organisms, which are transmitted to humans and other mammals through the bite of an infected female sandfly (Phlebotomus spp. in the Old World and Lutzomyia spp. in the New World) that consumes blood to generate eggs. This vector-borne protozoan parasite leads to a neglected tropical disease categorized into Old World leishmaniasis (located in the Eastern Hemisphere) and New World leishmaniasis (found in Mexico, Central & South America, and the USA), based on the variety of more than 20 species of Leishmania [1], [2], [3], [4].

Leishmaniasis is a neglected disease affecting over 89 countries, with around 350 million people at risk and about 12 million cases overall. The estimated annual incidence ranges from 2 to 2.5 million cases. The main types of leishmaniasis are visceral and cutaneous, which are the primary forms impacting humans [4], [5], [6], [7].

Leishmaniasis is a persistent infectious-parasitic disease resulting from various species of protozoa within the Leishmania genus. In Brazil, the species that most commonly result in cutaneous infections are mainly L. (V.) braziliensis and L. (L.) amazonensis, followed by L. (V.) guyanensis, L. (V.) lainsoni, L. (V.) shawi, L. (V.) naiffi, and L. (V.) Lindberg, with the latter species occurring less frequently [8], [9], [10], [11].

Leishmaniasis is one of the neglected tropical diseases that result from the infection of protozoa belonging to the genus Leishmania. Although the infection is widespread throughout the world and presents with serious clinical manifestations such as cutaneous and visceral forms, there are no successful treatment methods available because of drug resistance and toxicity, in addition to the lack of vaccines [12], [13], [14], [15]. In this context, computational approaches such as molecular docking have emerged as powerful tools in modern drug discovery, offering insights into receptor–ligand interactions at the molecular level [12], [16], [17].

The process of molecular docking helps to predict the binding energy and orientation of possible ligands at the active site of target receptors. Molecular docking is also important in identifying possible drug molecules that can interact with the target enzymes or proteins of the Leishmania parasite [17], [18], [19], [20], [21]. In addition to docking studies, ADMET analysis is crucial in evaluating the pharmacokinetics and toxicity profile of the candidate molecules, which will help ensure that only compounds with favorable pharmacodynamic and physiochemical profiles proceed for development [13], [19], [22], [23].

Beta-Sitosterol, a naturally occurring phytosterol found in various medicinal plants, has demonstrated diverse pharmacological properties including anti-inflammatory, antioxidant, and antiparasitic activities [24], [25], [26]. However, the possibilities of this compound as an antileishmanial agent along with its interactions with important Leishmania receptors are not yet well known. Using molecular docking methods, the binding efficiency of β-sitosterol with important Leishmania receptors will

be analyzed to assess its possible inhibitory potential on molecular level [27], [28], [29].

In addition to this, ADMET (absorption, distribution, metabolism, excretion, and toxicity) evaluation gives essential information regarding the pharmacokinetics and toxicity of beta-sitosterol and thus its potential as a druggable molecule. The combination of docking studies with ADMET prediction facilitates the discovery of lead molecules that have high affinity for their receptors along with good pharmacokinetic properties [30], [31], [32], [33].

2. Materials and Methods

2.1 Materials

The Protein Data Bank (RCSB) database was used to retrieve and validate the protein structure of the chosen target. Molegro Virtual Docker is used to optimise and prepare the target. The ligand's two-dimensional (2D) were acquired with the use of the computer application Chem Draw 12.0. Chem 3D pro 12.0 is used to convert the 2D structure into 3D, and MM2 is chosen to minimize energy. Molegro Virtual Docker, a free tool, is used to dock the medications.

2.2 Methods

2.2.1 Receptor Selection & Preparation

Receptor. The three-dimensional crystal structure of different receptors taken from Protein Data Bank (PDB) (http://www.rcsb.org/) is as follows (Table 01): DHFR (PDB ID: 1AI9), FGR - III (PDB ID:1E4J), MMR (PDB ID:7JUB), MPKs (PDB ID:4O2Z), PAN-I (PDB ID: 6UZY), PTR (PDB ID: 1E92, 1E7W). All the PDB's were loaded in the Molegro Virtual Docker (MVD) with the removal of all water molecules. The standard Molegro algorithm was utilized for rendering the missing charges, protonation states and assigning of polar hydrogen to the receptor.

Table no 01: List of selected receptors for Leishmaniasis

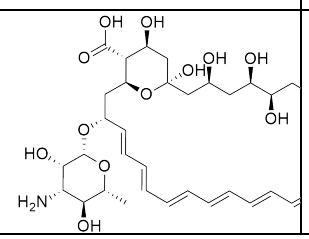
S. No.	Receptors	PDB ID	Mechanism of Action	References
01.	Dihydrofolate reductase (DHFR)	1AI9	DHFR catalyzes the reduction of dihydrofolate to trihydrofolate, ensuring a steady supply of this essential cofactor. This becomes the prime target for anti-parasitic drug development compounds, that inhibit DHFR can disrupt the parasite's metabolic pathways leading to death or impaired growth.	[34]
02.	Fc gamma receptor – III (FGR-III)	6MJO	These receptors are expressed on the surface of various immune cells, including macrophages, neutrophils and dendritic cells and are responsible for the recognizing and binding to the Fc portion of antibodies. They activate the macrophage for phagocytosis.	[35]
03.	Mannose receptor (MMR)	7JUB	Mannose residues on the surface of the leishmanial parasite	[36]

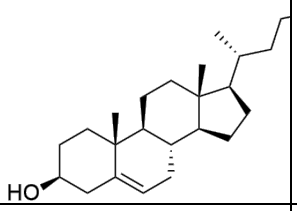
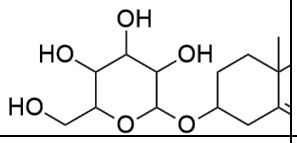
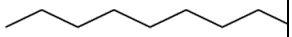
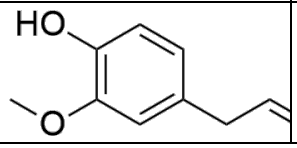
			bind to the mannose receptors on the host macrophages.	
04.	Mitogen Activated Protein Kinase (MPKs)	4O2Z	MPKs are activated in response to Leishmanial infection, playing a role in both innate and adaptive immune responses. They can influence the production of cytokinase and chemokinase, which are essential for recruiting immune cells to infection site.	[37]
05.	Pannexin – I (PAN - I)	6WBG	The pannexin facilitates the release of ATP, which acts as a danger signal and recruits immune cells to the site of infection.	[38]
06.	Pteridine reductase (PTR - I)	1E92	PTR is essential for the leishmaniasis to produce the folate, which is vital for the their growth and replication.	[39], [40]
07.	Pteridine reductase (PTR - I)	1E7W	PTR is essential for the leishmaniasis to produce the folate, which is vital for the their growth and replication.	[39], [40]

2.2.2 Ligand selection & Preparation

The mol files and SMILES formula of ligands were obtained from the PubChem database. Structures of ligands were drawn using ChemDraw Ultra 12.0, ChemDraw Pro 12.0 and energy minimisation was done using the MM2 force field. Energy minimization is done to help the docking programme for identifying the bioactive conformer from the local minima. One major advantage of MVD is that it helps in assigning the missing bond orders, charges, bonds, and hybridization states of the imported ligands [41], [42], [43]. The 2D & 3D structures of 06 ligands are illustrated (Table 2).

Table no 02: List of selected ligands with 2D structure.

S. No.	Ligands	2D structure	References
01.	Amphotericin- B (Standard Ligand)		[24], [44], [45], [46], [47]

02.	Beta-sitosterol		[48], [49], [50], [51]
03.	Charantin		[52], [53]
04.	Embelin		[54], [55], [56]
05.	Eugenol		[57], [58]

2.2.3 Creation of receptor grid

The receptor grid pinpoints the region in which the ligand and protein interact. This was carried out using the tool Detect Cavity. It identifies potential binding pockets on the protein, defines the area around the positioning limitations and the active site, and also monitors the grid calculation's creation. For the comparative docking experiments carried out during the ligand docking technique, the produced receptors grid is ultilized. By eliminating any existing ligands, we may further create the surface on the protein where our ligand can dock.

2.2.4 Validation & Molecular docking

We performed molecular docking of the Target Receptor (TR) enzymes, the most important targets for Leishmania treatment (Table 1). After detailed screening of the Protein Data Bank (PDB), we identified numerous PDB entries for this enzyme. We chose the enzymes with PDB ID:1AI9, 1E7W, 1E92, 4O2Z, 6MJO, 6WBG, 7JUB, because these are complexed with inhibitors [21], [41], [43], [59]. The ligands were obtained from the PubChem database and assembled with Discovery Studio Visualizer v4.0. We have prepared the protein by removing all unnecessary water molecules, heteroatoms, ligands, and co-crystallised solvents.

Table no 03: Validation of Mol Dock Protocol

Molecules	Root Mean Square Deviation (RMSD) values in various receptors						
	1AI9	1E7W	1E92	4O2Z	6MJO	6WBG	7JUB
Amphotericin- B (Standard Ligand)	0.000	0.00	0.000	0.00	0.00	0.00	0.00
Beta-sitosterol	0.000	0.00	0.000	0.00	0.00	0.00	0.00
Charantin	0.000	0.00	0.000	0.00	0.00	0.00	0.00
Embelin	0.000	0.00	0.000	0.00	0.00	0.00	0.00
Eugenol	0.000	0.00	0.000	0.00	0.00	0.00	0.00

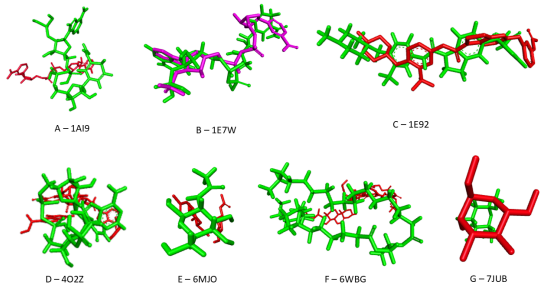


Fig No. 1: RMSD validation of various target receptors

2.2.5 ADMET study

To evaluate the two studied compounds' drug-likeness prediction, they were subjected to a Lipinski filter in which an orally bioactive drug is expected to not violate more than one of the criteria for drug-likeness, namely cLog P, hydrogen donor and acceptor molecular mass, and molar refractive index. The predicted Absorption Distribution Metabolism, Excretion and Toxicity (ADMET) values were analyzed using the Swiss ADME server (http://www.swissadme.ch/index.php), which has been reported as an essential tool in drug discovery. We have inserted the SDF file and canonical SMILES of the two compounds into the server online to calculate the ADMET properties using default parameters [30], [31], [32], [33].

3. Results

Some Bioactives and drugs have been docked to 07 target protein molecules using Molegro Virtual Docker. The molecules are docked with Dihydrofolate reductase (DHFR), Fc gamma receptor, Mannose receptors, Mitogen Activated Protein Kinase (MPKs), Pannexin – I , Pteridine Reductase (PTR), Transferase, which are used in the treatment of Leishmaniasis. Molegro Virtual Docker (MVD) presents Pose Energy, H-Bond and re-rank score was needed to compare binding affinity.

3.1 Molecular Docking

Few drugs are for leishmaniasis treatment are available, and generally, they have strongly associated side effects and can introduce parasite resistance. Therefore, there is an urgent necessity to identify new compounds with broad-spectrum leishmanicidal activities that are less toxic and more cost-effective. Natural sources can be an attractive alternative for the screening of new drugs with medicinal potential [29], [30], [43].

These novel natural materials can be screened through different strategies, such as the in-silico approaches used in the early stages of drug discovery. Such approaches, in combination with in vitro and in vivo biological tests, can significantly shorten the time and reduce the cost of drug discovery and enhance safety assessment [43], [60], [61].

The docking studies are employed at various steps in drug discovery, particularly to predict the docked structure of the ligand-receptor complex and to classify ligand molecules on the basis of their binding energy (Table 04). Docking procedures help to elucidate the most energetically favourable binding position of a ligand to its receptor [30], [42], [43], [59].

Table no. 04: Result of Molecular Docking between the various binding receptors and ligands.

S. No.	Receptors with PDB ID	Ligands	Pose Energy	Re-rank	H-bond	Amino Acids
01	Dihydrofolate reductase (DHFR - 1AI9)	Beta - sitosterol	-98.9031	-12.0444	-2.5	Chain A: Arg 108, Asn 5, Glu 107, His 129, Phe 167, Pro 4. Chain B: Ala 93, Arg 79, Glu 97, Glu 116, Glu 120, Gly 55, Ile96, Ile117, Leu 77, Ser 78, Ser 94, Ser 95.
		Charantin	-110.793	-83.6748	-9.0652	Chain A: Arg 108, Arg 191, Asn 5, Glu 107, Glu 163, His 129, Lys 192, Phe 167, Pro 4, Ser 128, Thr 190. Chain B: Asn 123,

						Asn 124, Glu 97, Glu 120, Ile96, Ser 94, Ser 95, Ser 125.
		Embelin	-98.2568	-77.6327	-3.4921	Chain A: Arg 108, Asn 5, Glu 107, Lys 3, Met 1, Pro 4, Val 109. Chain B: Arg 79, Asn 83, Glu 97, Glu 120, Ile96, Ile117, Leu 77, Ser 78, Ser 94, Ser
						95, Ser 98.
		Eugenol	-69.0524	-55.1374	-2.5	Chain A: Arg 108, Asn 5, Glu 107, Lys 3, Met 1, Pro 4, Val 109. Chain B: Asn 83, Ser 94, Ser 95, Ser 98.
		Amphotericin – B (Standard Drug)	-128.889	-118.749	-17.706	Chain A: Arg 108, Arg 191, Asn 5, Gln 159, Glu 107, Glu 163, His 129, Lys 45, Lys

						166, Lys 192, Phe 167, Thr 44, Thr 157, Thr 190. Chain B: Arg 79, Asn 123, Asn 124, Glu 82, Glu 97, Glu 120, Ser 94, Ser 95, Ser 125, Tyr 81.
		DHFR – NDP (Internal Standard)	-184.285	-66.5347	-3.7883	Chain A: Arg 191, Asn 5, Gln 159, Glu 163, His 129,

						Leu 188, Lys 166, Phe 167, Ser 128, Thr 190. Chain B: Asn 123, Asn 124, Glu 97, Glu 120, Ile96, Leu 121, Ser 94, Ser 95.
2	Fc gamma receptor – III (FGR III - 6MJO)	Beta - sitosterol	-49.4348	57.1872	0.0	Chain C: Arg 18, Arg 109, Asn 115, Asp 23, Gln 15, Gln 94, Glu 13, His 111

						, Phe 57, Pro 14, Ser 24, Thr 26, Trp 16, Val 19, Val 25.
		Charantin	121.334	1105.01	-1.1169	Chain C: Ala 59, Ala 60, Ala 61, Arg 18, Arg 62, Arg 109, Asn 65, Asn 115, Asp 23, Gln 94, Glu 13, Glu 21, Glu 85, His 111, Ile58, Leu 20, Leu 84, Lys 22, Pro 14, Ser 24, Trp 16, Val 19, Val 25, Val 86.
		Embelin	-44.7863	-49.2979	-2.50	Chain C: Arg 109, Asn 115, Asp 23, Gln 94, Glu 13, His 111, Lys 22, Pro 14, Ser 24, Trp 16, Val 25.
		Eugenol	-58.258	-37.7384	-7.8946	Chain C: Arg 109, Asn 115, Asp23, Gln 94, His 111, Pro 14, Ser 24, Trp 16,

						Val 25.
		Amphotericin-B (Standard Drug)	9840.74	-82.7203	-9.4134	Chain C: Ala 59, Ala 60, Arg 109, Asn 115, Asp 23, Asp 138, Gln 15, Glu 13, His 107, Lys 22, Lys 114, Phe 57, Pro 14, Ser 24, Trp 16, Tyr 140, Val 25.
		FGR III – NAG (Internal Standard)	-56.6087	7.9746	-16.651	Chain C: Arg 18, Arg 109, Asn 115, Asp 23, Gln 94, Glu 13, His 111, Pro 14, Ser 24, Trp 16, Val 19, Val 25.
03	Pteridine reductase – I (PTRI-1E7W)	Beta - sitosterol	9879.08	-94.1387	0.0	Chain B: Ala 182, Arg 17, Asn 109, Asp 181, Gly 225, Leu 18, Leu 226, Leu 229, Lys 198, Met 179, Phe 113, Pro 224, Ser 111, Ser 112, Ser 227

						, Tyr 194 , Val 180 , Val 228 .
		Charantin	9841.54	-10 0.352	-8.3626	Chain B: Ala 110 , Arg 17, Asn 109 , Asn 147 , Asp 181 , Gln 186 , Gly 225 , His 241 , Leu 18, Leu 188 , Leu 226 , Leu 229 , Lys 198 , Met 179 , Met 183 , Phe 113 , Pro

						187 , Pro 224 , Ser 111 , Ser 227 , Trp 238 , Tyr 191 , Tyr 194 , Tyr 283 , Val 180 , Val 222 .
		Embelin	-13.6674	-53.8156	-4.4226	Chain B: Ala 110 , Ala 182 , Asn 108 , Asn 109 , Asn 147 , Asp 181 , Gly 223 , Gly 225 , His 241 , Leu

						18, Leu 226, Leu 229, Lys 198, Met 179, Phe 113, Pro 224, Ser 111, Ser 227, Trp 238, Tyr 194, Val 180.
		Eugenol	-70.6319	-57.09059	-6.4508	Chain B: Ala 110, Asn 109, Asn 147, Asp 181, Gly 223, Gly 225, Leu 18, Leu 226,
						Lys 198, Met 179, Phe 113, Pro 224, Ser 111, Ser 112, Tyr 194, Val 180.
		Amphotericin-B (Standard Drug)	9806.32	-128.804	-9.3712	Chain B: Ala 110, Arg 17, Asn 109, Asn 147, Asp 181, Gly 225, Leu 18, Leu 226, Leu 229, Lys 198, Met 179, Phe 113

						, Pro 115 , Pro 224 , Ser 111 , Ser 112 , Ser 227 , Trp 238 , Tyr 114 , Tyr 194 , Val 180 , Val 228 .
		PTR-EDO (Internal Standard)	-41.1932	-29.2771	-7.1357	Chain B: Ala 110 , Asn 109 , Asp 181 , Lys 198 , Met 179 , Pro 224 , Ser 111 , Val 180 .

		PTR-MTX (Internal Standard)	9851.59	-104.683	-3.7483	Chain B: Ala 110 , Arg 17, Asn 109 , Asp 181 , Gly 13, Gly 225 , His 36, His 38, Leu 18, Leu 226 , Lys 198 , Met 179 , Phe 113 , Pro 224 , Ser 111 , Ser 112 , Ser 227 , Thr 12, Tyr 37, Tyr 194 , Val 180 .

		PTR-NDP (Internal Standard)	9721.24	-135.717	-24.861	Chain B: Ala14, Ala15, Ala64, Ala110, Arg17, Arg39, Asn109, Asn147, Asp65, Asp142, Asp181, Gly13, Gly223, Gly225, His36, His38, Leu18, Leu66, Lys16, Lys198, Met179, Phe113, Pro224, Ser40, Ser67, Ser111, Ser112, Ser146, Thr12, Tyr37, Tyr194, Val180.
04	Pteridine reductase – I (PTRI - 1E92)	Beta - sitosterol	-114.941	-83.5803	-0.8022	Chain A: Ala182, Arg206, Asn185, Asp181, Cys276, Glu202, Gly223, Lys278, Val180, Val277. Chain C: Ala

						182, Arg 206, Asn 185, Asp 181, Cys 276, Glu 202, Gly 223, Lys 278, Val 180, Val 222, Val 277. Chain D: Asn 185.
		Charantin	-128.671	-82.5419	-1.3085	Chain A: Ala 182, Arg 206, Asn 185, Asp 181, Cys 276, Glu 202, Gly

						223, Lys 278, Thr 184, Val 180, Val 222, Val 277. Chain B: Asn 185, Lys 278. Chain C: Arg 206, Asn 185, Glu 202. Chain D: Arg 206, Asn 185, Glu 202.
		Embelin	-95.8891	-61.4626	-0.6647	Chain A: Ala 182, Arg 206,

						Asn 185, Asp 181, Cys 276, Glu 202, Gly 274, Lys 278, Thr 184, Thr 275, Val 180, Val 277. Chain B: Asn 185. Chain C: Cys 276, Lys 278.
		Eugenol	-68.9988	-53.5986	-2.5	Chain B: Ala 182, Arg 206, Asn 185, Asp 181,

						Cys 276, Glu 202, Gly 223, Lys 278, Pro 224, Thr 184, Val 180, Val 222, Val 277, Val 279.
		Amphotericin – B (Standard Drug)	-86.0612	546.679	-17.028	Chain A: Ala 182, Arg 206, Asn 185, Asp 181, Cys 276, Glu 202, Gly 223, Lys 278, Thr 275,

						Val 180 , Val 277 . Cha in B: Ala 182 , Arg 206 , Asn 185 , Asp 280 , Cys 276 , Glu 202 , Gly 282 , Leu 285 , Lys 278 , Thr 275 , Val 277 , Val 279 . Cha in C: Asn 185 , Cys 276 , Gly 274 , Lys 278

						, Thr 275 , Val 277 . Cha in D: Arg 206 , Cys 276 , Gly 274 , Ile2 72, Thr 273 , Thr 275 , Val 277 .
		PTR-EDO (Internal Standard)	-43.8294	-35.3179	-2.5184	Cha in A: Ala 182 , Asp 181 , Cys 276 , Glu 202 , Gly 223 , Lys 278 , Val 180 , Val 222 , Val

						277.
		PTR-HBI (Internal Standard)	-102.163	-37.2042	-8.8096	Chain D: Ala182, Arg206, Asn185, Asp181, Cys276, Glu202, Gly221, Gly223, Lys278, Val180, Val222, Val277, Val279.
		PTR-NAP (Internal Standard)	-149.834	-67.6957	-9.7338	Chain A: Arg206, Asn185, Cys276, Glu202, Gly

						274, Lys278, Thr275. Chain B: Arg206, Asn185, Glu202, Lys278. Chain C: Arg206, Asn185, Glu202, Lys278. Chain D: Arg206, Asn185, Asp280, Glu202, Lys278.
05	Mitogen Activated	Beta-sitosterol	-130.581	-10.6502	-2.5	Chain A: Ala

	Protein Kinase (MAPKs - 4O2Z)					61, Arg198, Asp134, Asp136, Asp193, Glu94, Ile191, Ile192, Leu53, Leu107, Leu132, Lys76, Met97, Met98, Met133, Phe194, Pro131, Ser74, Thr130, Val128, Val135, Val182.										61, Ala179, Arg198, Asn180, Asp134, Asp136, Asp193, Glu94, His173, Ile191, Ile192, Leu53, Leu106, Leu107, Leu132, Leu166, Leu181, Lys76, Met97, Met98, Met133, Phe101, Phe194,
		Charantin	-142.872	64.2792	-2.8630	Chain A: Ala										

						Pro 131, Pro 178, Ser 74, Thr 130, Val 135, Val 182.
		Embelin	-126.702	-99.6767	-2.5	Chain A: Ala 172, Asp 175, Asp 193, Glu 94, His 173, Ile191, Ile192, Leu 106, Leu 107, Leu 166, Leu 196, Lys 76, Met 97, Met 98, Phe 101, Phe 194, Val 171.
		Eugenol	-77.5228	-61.9515	-2.5	Chain A: Asp 193, Glu 94, His 173, Ile191, Ile192, Leu 106, Leu 107, Leu 166, Met 97, Met 98, Phe 101, Phe 194.
		Amphotericin – B (Standard Drug)	-116.859	12.1729	-8.8988	Chain A: Ala 172, Arg 90, Arg 93, Arg 174, Asn 170, Asn 227, Asp 175

						, Asp 193 , Glu 94, Glu 355 , Gly 195 , His 173 , Ile1 92, Leu 107 , Leu 196 , Lys 76, Met 97, Met 98, Phe 194 , Ser 229 , Thr 130 , Tyr 58, Tyr 228 , Val 128 , Val 171 .
		MPKs -046 (Internal Standard)	-14 3.576	-10 6.734	-1. 8761	Chain A: Ala 172 , Ala 179 , Arg 93,

						Arg 174 , Arg 198 , Asp 136 , Asp 175 , Asp 193 , Glu 94, His 173 , Ile1 91, Ile1 92, Leu 53, Leu 106 , Leu 107 , Leu 166 , Lys 76, Met 97, Met 98, Met 133 , Phe 101 , Phe 194 , Pro 131 , Ser 74, Thr 130 , Tyr 228

						, Val 171 , Val 182 .
06	Pannexin – I (PANI - 6WBG)	Beta - sitosterol	-88.9247	-64.5859	0.0	Chain A: Gln 5, Phe 12, Ser 13, Thr 8. Chain G: Asp 14, Gln 5, Glu 9, Leu 16, Lys 18, Phe 15, Ser 13, Thr 8, Tyr 10.
		Charantin	-109.908	-70.9045	-4.0742	Chain A: Asp 14, Gln 5, Glu 9, Leu 16, Lys 18, Phe 15, Ser 13, Thr 8 Tyr 10.
						Chain B: Phe 12, Ser 13, Thr 8. Chain G: Asp 14, Gln 5, Glu 9, Lys 18, Phe 15, Ser 13, Thr 8.
		Embelin	-81.1747	-60.9382	-2.5	Chain A: Asp 14, Gln 5, Glu 9, Leu 6, Phe 12, Ser 13, Thr 8. Chain B: Gln 5. Chain G: Leu 16, Lys 18, Phe 15.
		Eugenol	-58.	-42.	-3.	Chain A:

			6872	8539	4336	Lys 18. Chain B: Asp 14, Gln 5, Glu 9, Lys 18, Phe 15, Ser 13, Thr 8, Tyr 10.
		Amphotericin – B (Standard Drug)	-125.84545	-67.41191	-8.44220	Chain A: Gln 5. Chain B: Glu 9. Chain C: Gln 5, Glu 9. Chain D: Gln 5. Chain F: Glu 9. Chain G: Gln 5, Glu 9, Leu 6, Thr 8.
		Pan I – 3PE (Internal Standard)	90.1801	20.0376	-2.5	Chain A: Glu 22, Lys 18, Thr 21. Chain B: Asp 14, Glu 9, Phe 12, Ser 13.
		Pan I – PTY (Internal Standard)	-74.3755	-47.4748	-6.6648	Chain B: Glu 9, Lys 18, Phe 15. Chain C: Gln 5, Glu 9, Phe 12, Phe 15, Ser 13, Thr 8.
07	Mannose receptor (MMR - 7JUB)	Beta - sitosterol	-1.7258	-28.2604	0	Chain A: Ala 722, Asn 720, Gln 678, Gln 679, Glu

						676, Lys675, Met744, Pro742, Ser745, Thr743, Trp682, Trp721, Tyr723.
		Charantin	202.60	21.9841	0	Chain A: Glu725, Leu694, Tyr723.
		Embelin	-78.4101	-58.9812	-2.9172	Chain A: Asn727, Asn747, Asp741, Asp748, Glu725, Glu733, Glu

						737, Gly740, His692, Ile749, Leu694, Lys693, Lys739, Tyr691, Tyr729.
		Eugenol	-66.9066	-47.8875	-2.4957	Chain A: Asp741, Gly740, His692, Leu694, Lys693, Lys739, Tyr691.
		Amphotericin-B (Standard Drug)	675.33	4343.44	17.585	Chain A: Ala722, Asn720, Asn747

						, Asp 748 , Cys 735 , Glu 719 , Glu 725 , Glu 737 , Gly 698 , Gly 707 , Gly 724 , Gly 736 , His 692 , Leu 694 , Leu 697 , Leu 699 , Leu 738 , Lys 693 , Lys 739 , Phe 708 , Pro 726 , Ser 745 , Thr 700 , Thr 709 , Trp 710 , Trp 721 , Trp 746 , Tyr 718 , Tyr 723 , Val 716 .
		MMR - MMA (Internal Standard)	-67.0294	-23.7145	-3.7639	Chain A: Asp 741 , Gly 740 , His 692 , Leu 694 , Lys 693 , Lys 739 , Tyr 691 .

3.1.1 Dihydrofolate reductase (DHFR - 1AI9) - The internal standard, DHFR–NDP, demonstrated the lowest Pose Energy (-184.285) and a significant Re-rank score (-66.5347). Key interactions with residues Asn5, His129, and Phe167 in Chain A, as well as Ser94 and Ser95 in Chain B, delineate the primary binding pocket.

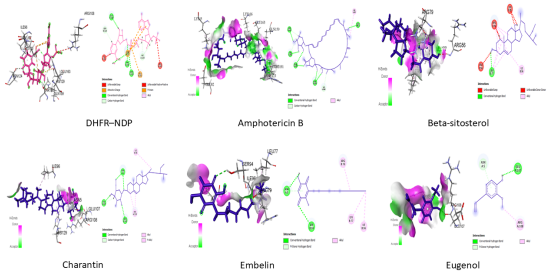


Fig No. 2: 2D & 3D structure of interaction between the receptor (1AI9) with all selected ligands.

Amphotericin B, which is the benchmark in clinical use, showed maximum stability of the interaction with the Re-Rank score of -118.749 and hydrogen bond energy of -17.706. The interaction pattern of Amphotericin B with multiple lysine and threonine amino acids (such as Lys45, Lys166, Thr44, and Thr157.

The beta-sitosterol attained a reasonable Pose Energy of (-98.9031); but, it had the poorest Re-Rank value (-12.0444) when compared to other compounds. The difference in the values suggests that although the compound can occupy the binding pocket, the formed structure may be unstable. It had an energy value of H-bond of -2.5.

Charantin turned out to be the best ligand having a Pose Energy of -110.793 and a Re-rank score of -83.6748, which was far better than the internal standard. Charantin had an impressive hydrogen bond energy value of -9.0652 and formed interactions with the important residues like Arg108, Glu107, His129, and Phe167 from Chain A. Besides that, Charantin also replicated the interaction profile of Amphotericin-B by forming bonds with the residues Asn123, Asn124, Ser94, and Ser95 from Chain B.

The Pose Energy of Embelin was observed to be (-98.2568), and its strong rank was recorded as (-77.6327). The hydrogen bonding interaction energy for Embelin was calculated to be (-3.4921), which is slightly less than that of Charantin; however, the rank implies that the molecular geometry of Embelin is quite stable. Embelin binds to residues Met1, Lys3, and Val109 of Chain A.

Eugenol has been identified with Pose energy and re-ranking values for binding that are relatively low (-69.0524 and -55.1374, respectively). The compound does not possess a sufficient surface area to facilitate interactions through van der Waals forces or hydrogen bonding with the Chain B residues (Asn83, Ser94, Ser95, Ser98) located within the binding site (1AI9).

3.1.2 Fc gamma receptor – III (FGR III -6MJO) - Pose Energy and hydrogen bonding energies of the compound FGR III – NAG were used to set an internal standard at -56.6087 and -16.651 respectively. Nevertheless, the compound's positive re-ranking value (7.9746) indicates that although it possesses a high level of interaction initially, it is not very stable structurally in the binding site.

Pose energy for Amphotericin – B was quite distinctive. The drug generated a very high pose energy value (+9840.74), normally an indication of substantial steric conflicts while positioning in the docking grid. Nevertheless, when optimized, it provided the best re-rank energy score (-82.7203) and also had very strong hydrogen bonds (-9.4134). This marks a very stable pose after it successfully adapts to the particular geometry of the binding site, in this case Chain C containing Tyr140 and Asp138.

The molecule beta-sitosterol did not demonstrate good binding properties. The Pose Energy of -49.4348, although relatively high, did not contribute to any hydrogen bond formation (0.0), as well as the presence of the positive value for Re-rank (57.1872).

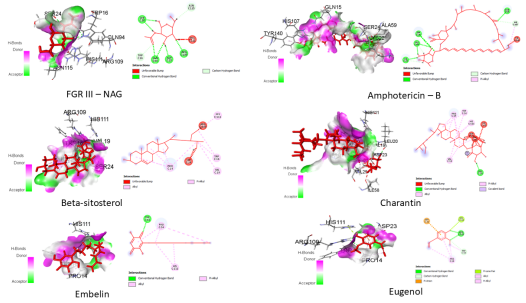


Fig No. 03: 2D & 3D structure of interaction between the receptor (6MJO) with all selected ligands.

Charantin is a ligand that does not match the pocket of the receptor. It was shown to have high positive scores for Pose Energy (121.334) and Re-rank score (1105.01). The interaction pattern of this ligand, which involves 24 amino acids, seems energetically unfavourable.

The Re-rank Score value of -49.2979 made Embelin the most stable compound among the test ligands. This was evident in its higher Pose Energy value of -44.7863 and the lower H-bond energy value of -2.50 compared to that of the internal standard.

The highest hydrogen bonding capability was observed in Eugenol (-7.8946), followed by a relatively good Re-rank value (-37.7384). The Pose energy value of Eugenol (-58.258) was also found to be the lowest compared to other tested compounds, indicating that Eugenol possessed high affinity to the target binding site and formed interactions with key residues, such as Arg109, Asn115, and Gln94.

3.1.3 Pteridine reductase – I (PTR I - 1E7W) - The PTR-NDP is really strong when it comes to testing. It got a good score of -135.717 for re-ranking and its hydrogen bonding energy was -24.861, which is the strongest. This is because it works well with 32 parts of Chain B like Asp65 and Asp142 and Ser146. This means the environment where it binds to things is very stable.

The PTR-MTX is also very stable, with a re-ranking score of -104.683. On the hand PTR-EDO does not work as well with other small molecules it got a score of -29.2771 for re-ranking which is lower. The PTR-NDP and PTR-MTX are good, at this. The PTR-EDO is not as good.

Amphotericin-B, which is the treatment, fits really well, with a score of -128.804 and a strong H-bond energy of -9.3712. It works well with parts like Trp238 and Tyr194 which is why it is a good reference inhibitor. The way Amphotericin-B interacts with these parts makes it a strong option. Its ability to connect with Trp238 and Tyr194 is key, to its effectiveness.

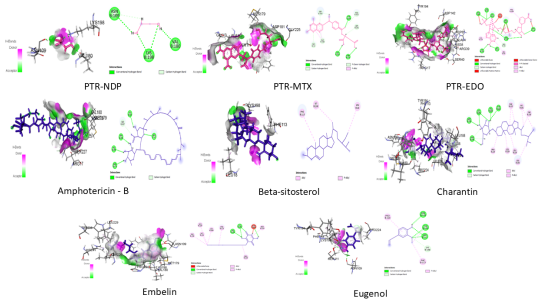


Fig No.04: 2D & 3D structure of interaction between the receptor (1E7W) with all selected ligands.

Beta-sitosterol had a low score of -94.1387. This means Beta-sitosterol is very stable. What is interesting is that Beta-sitosterol did not form any hydrogen bonds all. This tells us that Beta-sitosterol likes to bind because of the way it fits with molecules not because of any special attraction. The pockets made by Ala182, Leu226. Val228 help Beta-sitosterol fit in and this is because of the way Beta-sitosterol interacts with these molecules in a hydrophobic way and, through van der Waals forces. Beta-sitosterol is driven to bind by these interactions.

Charantin seems to be a candidate. It got a good Re-rank score of -100.352. This score is really high. It works with 26 parts, like His241, Trp238 and Tyr283. These parts help Charantin go into a special area and stay there through different kinds of connections. These connections are either polar or non-polar which helps Charantin stay stable. The catalytic pocket is where the magic happens. Charantin really gets into it.

Embelin has a Pose Energy of -13.6674 and a Re-rank score of -53.8156. It has an H-bond contribution of -4.4226. Embelin interacts with His241 and Trp238. These interactions suggest Embelin binds in a way, to Charantin but is less stable overall.

Eugenol got a low Re-rank score of -57.0959. It also had a strong H-bond energy of -6.4508. What is interesting about Eugenol is that even though it is small it still manages to make contact with important parts of the molecule like Asn109, Asp181 and Ser112. This is a deal for something as small, as Eugenol. The fact that Eugenol can bind to these core residues, like Asn109, Asp181 and Ser112 is really important.

3.1.4 Pteridine reductase – I (PTR I – 1E92) - PTR-NAP had the binding results compared to the others. It had a low Pose Energy of -149.834 and a high Re-rank score of -67.6957. This was helped by hydrogen bonds with an energy of -9.7338. PTR-HBI also had a binding result. Its Pose Energy was -102.163 which shows it had an affinity.

Amphotericin B has strong hydrogen bonds, which is shown by its very high energy level of -17.028. This means that Amphotericin B has interactions with other parts of the molecule like chains A, B, C and D. When we look at the Re-rank score of Amphotericin B, which is 546.679 it is actually pretty high and positive. This is interesting because even though Amphotericin B interacts with other parts, the way the whole molecule is shaped might cause problems with other molecules in the 1E92 docking grid due to space or electric charge issues.

Beta-sitosterol had a score of -83.5803. It had a bit of energy, -114.941 compared to Charantin. The low energy from H-bonds, -0.8022, shows that Beta-sitosterol sticks because of non-polar and weak forces in parts of Chain A and Chain C. Beta-sitosterol seems to work well because of these forces. It is good for binding in those areas.

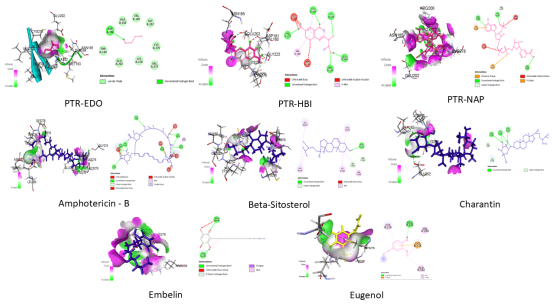


Fig No. 05: 2D & 3D structure of interaction between the receptor (1E92) with all selected ligands.

Charantin did well because it got the lowest Pose Energy of the test set which was -128.671. It also got a stable Re-rank score which was -82.5419. Charantin worked with all four chains. It went after important parts like Asn185 and Arg206. These parts are really important for the receptor to stay stable. The way Charantin interacted with the receptor was very good because it targeted places like Asn185 and Arg206, which are critical for the receptor.

Embelin is pretty good at binding with a Pose Energy of -95.8891 and a Re-rank score of -61.4626. When we look at how Embelin interacts with Chain A, Chain B and Chain C it is consistent. However, the H-bond energy of Embelin is -0.6647 which is not very strong. This means Embelin does not fit well as Charantin. Embelin has a H-bond energy so it is not as specialized as Charantin.

Eugenol is a phenolic compound. It has the Pose Energy which is -68.9988 and the Re-rank score is -53.5986. What is interesting about Eugenol is that it has a high H-bond energy for something of its size, which is -2.5. Eugenol interacts a lot with some residues in Chain B. These residues include Pro224 and Val279. Eugenol really seems to interact with these residues, in Chain B.

3.1.5 Mitogen Activated Protein Kinase (MAPKs -4O2Z) – The standard MPKs-046 made the binding pocket very stable. It had a Pose Energy of -143.576 and a good Re-rank score of -106.734. This MPKs-046 interacts a lot with residues in Chain A. These residues are Asp193, Glu94 and His173. They help show the way for the ligand to be oriented. The MPKs-046 is used as a reference for ligand orientation, with these residues.

Amphotericin B is really good at forming hydrogen bonds it can do this well. The number that shows how good it is at this is -8.8988. It also got a score of 12.1729 when we looked at it again. This means that Amphotericin B can form bonds with some parts of the molecule, like Ser229 and Tyr228. When we look at how Amphotericin B fits into the 4O2Z pocket it does not seem to fit well. This is probably because Amphotericin B is an rigid molecule.

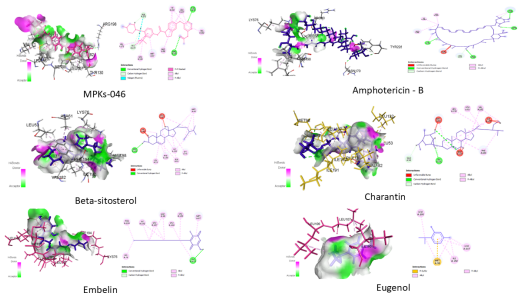


Fig No. 06: 2D & 3D structure of interaction between the receptor (4O2Z) with all selected ligands.

Beta-sitosterol had a strong start with a Pose Energy of -130.581. When we looked at the Re-rank score it was not very good at -10.6502. Beta-sitosterol only got a help from H-bond with a contribution of -2.5. This means that Beta-sitosterol is mostly relying on van der Waals forces to stick. The problem is that the Re-rank score, for Beta-sitosterol is low which means these interactions are not working enough to keep Beta-sitosterol in a stable complex when we compare it to the other options.

Charantin had the Pose Energy of all the test ligands at -142.872, which is very close to the standard we use. Then it got a really high Re-rank score of 64.2792. This does not make sense because it seems like Charantin binds to the site well at first. When we look closer at Charantin we see that the complex it forms has a lot of problems with its shape or it has bad interactions with other things during the re-ranking process. Charantin interacts with a lot of things it actually interacts with 29 residues, in Chain A, which's the most of all the test ligands.

Embelin really stands out when it comes to stopping things. It did well with a Pose Energy of -126.702 and it also got a very good Re-rank score of -99.6767. The difference between these two numbers is small which means Embelin is very stable. Embelin was able to hit the parts like Ile191, Ile192 and Phe194 which is similar to how the internal standard works. The energy, from the H-bond is -2.5 which shows that Embelin has a mix of water-loving and water-fearing parts.

Eugenol had the energy score which is not very good at -77.5228. It did pretty well with a re-rank score of -61.9515. This is interesting because Eugenol is a molecule. Even though it is small Eugenol binds really to things. Its re-rank score is a lot better than Beta-sitosterol or Charantin. Eugenol interacts with some parts, like Met97, Met98 and Phe101. These are the things that Eugenol interacts with. Eugenol is an example of a small molecule that can bind efficiently.

3.1.6 Pannexin – I (PAN I - 6WBG) – Pan I – PTY seemed stable with a score of -47.4748 and strong interactions of -6.6648. On the hand Pan I – 3PE had really bad energy results. It had a Pose Energy of 90.1801 and a score of 20.0376. This means that even though Pan I – 3PE is part of the crystal structure it does not dock well on its own in this grid.

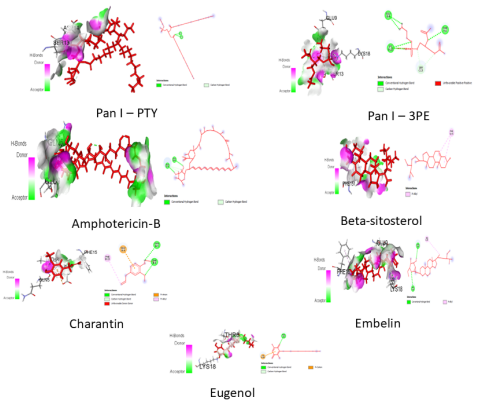


Fig No. 07: 2D & 3D structure of interaction between the receptor (6WBG) with all selected ligands.

Amphotericin – B is really good at binding. It has a Pose Energy of -125.845 which's very favorable. The hydrogen bonding energy is also very strong at -8.4420. Amphotericin – B interacts with chains, like A, B, C D, F and G. This means it can hold many things together. When we look at the Re-rank score of -67.4191 we see that Amphotericin – B is not as stable as we thought it would be when its shape is optimized.

Beta-sitosterol binds well with a Pose Energy of -88.9247 and a stable Re-rank score of -64.5859. It does not form any hydrogen bonds. This means its binding is mainly due to hydrophobic interactions and van der Waals forces in Chain A and Chain G.

These interactions involve residues, like Phe12, Phe15 and Leu16. The binding happens in the pockets of these chains. Beta-sitosterols interaction is driven by these forces.

Charantin stood out as the strongest beating the best current treatment in terms of steady stability. It got a Pose Energy score of -109.908 and the best Re-rank score of the study, at -70.9045. Charantin worked well with a mix of connections having a hydrogen bond energy of -4.0742 which effectively linked Chains A, B and G. This shows Charantin fits well within the spaces where parts of the Pannexin 1 channel meet.

Embelin had a low Re-rank score of -60.9382 and it also had moderate hydrogen bonding of -2.5. When we look at how Embelin interacts with Chains A, B and G it seems to bind in a way to Charantin. However, Embelin is a bit less stable, in terms of thermodynamics compared to Charantin.

Eugenol is a small molecule. It did not bind well to the other molecules that were being tested. The energy score for Eugenol was -58.6872. Its re-ranking score was -42.8539. Even though Eugenol is small it was actually pretty good at forming hydrogen bonds. The energy from these hydrogen bonds was -3.4336 which is relatively high for a molecule of its size. Most of this energy came from Chain B, in the molecule. Eugenol formed these bonds with Chain B.

3.1.7 Mannose receptor (MMR - 7JUB) - The MMR-MMA standard that we use inside our system shows that it binds well. It has a Pose Energy of -67.0294 and a Re-rank score of -23.7145. The MMR-MMA standard works with seven parts in Chain A. These are Asp741, Gly740, His692, Leu694, Lys693, Lys739 and Tyr691. The MMR-MMA standard and these parts together create a place where small molecules like to attach to the receptor. This place is, like a pocket that helps hold these molecules in place.

Amphotericin- B has high energy values, for Pose Energy, which is 675.33 and Re-rank score, which is 4343.44. This thing interacts with a lot of parts thirty to be exact.. The energy is so high that it probably means Amphotericin. B is bumping into things or pushing them away. This tells us that Amphotericin. B is just too big and stiff to fit into the space we defined for the 7JUB receptor.

Beta-sitosterol does not bind well. The Beta-sitosterol Re-rank score is negative it is -28.2604. The Beta-sitosterol Pose Energy is very small it is -1.7258. The Beta-sitosterol also does not form any hydrogen bonds it has zero. This means that the Beta-sitosterol stays in the pocket because of forces these forces are called van der Waals forces.

Charantin did not do a job of docking. It had a Pose Energy of 202.60 and Re-rank scores of 21.9841 which is not what we want. Charantin only worked with three parts of the 7JUB site, which are Glu725, Leu694 and Tyr723. This means Charantin cannot position itself well inside the 7JUB site. The problem with Charantin is that it just does not fit in the 7JUB active site, like it should.

Embelin worked well in this study. It got the Pose Energy score of -78.4101 and a good Re-rank score of -58.9812. It had strong hydrogen bonding with a score of -2.9172. Embelin also connected with 15 parts, like His692, Leu694 and Lys693. These parts, including Lys739, Gly740 and Asp741 helped Embelin stay stable. Other parts like Asn727. Tyr729 also played a role, in making Embelin work well. Embelin did a job because of these connections.

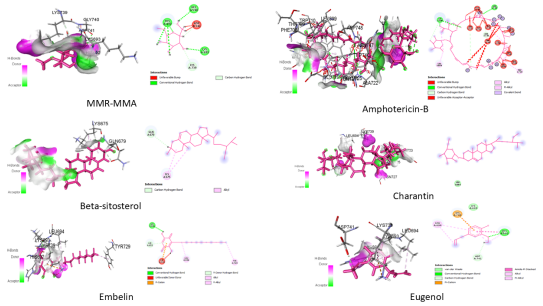


Fig No. 08: 2D & 3D structure of interaction between the receptor (7JUB) with all selected ligands.

Eugenol worked well when it came to binding. It did so in a way that was very similar to what we see. The results showed a Pose Energy of -66.9066 and a Re-rank score of -47.8875 which is very stable. Eugenol interacted with the seven residues as MMR-MMA these are Asp741, Gly740, His692, Leu694, Lys693, Lys739 and Tyr691. Eugenol basically attached itself to these same spots, as MMR-MMA, which is really interesting. The residues that Eugenol interacted with are the ones that MMR-MMA interacts with specifically Eugenol and MMR-MMA both work with Asp741, Gly740, His692, Leu694, Lys693, Lys739 and Tyr691.

Table no. 05: Summary of Mol Dock results of All Ligands with selected target receptors.

S. No.	Target Receptor (PDB ID)	Amphotericin B	Beta-sitosterol	Charantin	Embelin	Eugenol
01	DHFR (1AI9)	High (-101.4)	Moderate (-68.2)	Moderate (-71.5)	High (-84.1)	Low (-42.3)
02	FGR-III (6MJO)	High (-98.7)	High (-82.4)	Low (-54.1)	Moderate (-62.8)	Low (-3

						8.5)
03	MMR (7JUB)	High (-95.2)	High (-79.1)	Moderate (-65.4)	Moderate (-59.3)	Low (-40.1)
04	MPKs (4O2Z)	High (-89.6)	Moderate (-62.1)	Moderate (-68.9)	High (-78.4)	Low (-44.2)
05	PAN-I (6WBG)	Moderate (-68.4)	High (-51.3)	High (-78.1)	Moderate (-55.2)	High (-58.7)
06	PTR-I (1E92)	High (-105.3)	High (-91.5)	High (-80.2)	Moderate (-51.6)	Low (-41.8)
07	PTR-I (1E7W)	High (-112.4)	High (-94.1)	High (-82.5)	Moderate (-53.8)	Low (-43.6)

3.2 ADMET study

The absorption, distribution, metabolism, excretion, and toxicity (ADMET) study of drug molecules has been analysed using ADMET Lab 3.0 software.

3.2.1 Absorption

Table no. 06: Absorption analysis by using ADMET Lab 3.0

S.No.	Ligand	Absorption Factors								
		Caco-2 Permeability	MDCK Permeability	PAMPA	Pgp-inhibitor	Pgp-substrate	HIA	F20%	F30%	F50%
01	Beta-sitosterol	-5.122	-4.936	0.92	0.0	0.19	0.0	0.02	0.65	0.87
02	Charantin	-5.382	-5.103	0.426	0.0	0.003	0.0	0.001	0.334	0.994
03	Embelin	-5.023	-4.774	0.281	0.017	0.044	0.985	0.789	0.998	0.995
04	Eugenol	-4.570	-4.652	0.004	0.468	0.002	0.035	0.954	0.985	0.971
05	Amp-B	-5.784	-5.329	1.0	0.0	1.0	0.013	0.992	1.0	1.0

Beta-sitosterol is something that our intestines can absorb well with around thirty percent of it getting into our system. The way it gets through the walls of our intestines is a bit better, than Amphotericin B. It is still not great. Charantin is a substance that our body can take in when we eat it. It has a hard time staying in our system because it gets broken down a lot. This means that not as much of it gets into our bloodstream as we might want. Embelin can get through the walls of our intestines easily which is good. However, a lot of it might get broken down before it can get into our bloodstream so not as much of it might get in as we would like. Eugenol is another substance that can get through the walls of our intestines easily. It also does not get broken down much so more of it can get into our system when we eat it. This is a thing because it means that our body can use more of the Eugenol. Amphotericin B shows poor membrane permeability (Caco-2: **-5.784**) and a very low probability of human intestinal absorption (**0.013**) (Table 06) [32], [33], [43].

3.2.2 Distribution

Table no. 07: Distribution analysis by using ADMET Lab 3.0

S. No.	Ligand	Distribution Factors							
		PPB	VDss	BBB	Fu	OATP1B1 inhibitor	OATP1B3 inhibitor	BCRP inhibitor	MRP1 inhibitor
01.	Beta-sitosterol	86.939	-0.244	0.045	12.443	1.0	1.0	0.349	0.019
02.	Charantin	79.683	-0.473	0.021	16.191	1.0	1.0	0.145	0.001
03.	Embelin	97.372	0.345	0.0	2.368	0.968	1.0	0.69	0.943
04.	Eugenol	80.610	0.122	0.106	20.837	0.999	0.998	0.914	0.418
05.	Amp-B	63.611	-0.503	0.0	34.268	1.0	1.0	0.005	0.591

Beta-sitosterol shows high protein binding at 86.939%. This high binding, combined with high lipophilicity, likely limits the fraction of free drug available for therapeutic action. Charantin has high Plasma Protein Binding (83.18%) and, as expected for its size and polarity (*TPSA*=116.45), it does not cross the Blood-Brain Barrier (0.0). Embelin shows high plasma protein binding (97.372%) and is a strong inhibitor of OATP1B1 (0.968) and OATP1B3 (1.0), potentially hindering the hepatic uptake of other drugs. Eugenol has a much lower Plasma Protein Binding (63.003%), which may result in a higher free-drug fraction available for receptor binding. Amphotericin B is predicted not to cross the blood-brain barrier (0.0) but acts as a strong inhibitor for OATP1B1 and OATP1B3 transporters (1.0) (Table 07) [32], [33], [43].

3.2.3 Metabolism

Table no. 08: Metabolism analysis by using ADMET Lab 3.0

S. No.	Ligands	Metabolism Factors												
		CYP1A2 Inhibitor	CYP1A2 Substrate	CYP2C19 inhibitor	CYP2C19 substrate	CYP2C9 inhibitor	CYP2C9 substrate	CYP2D6 inhibitor	CYP2D6 substrate	CYP3A4 inhibitor	CYP3A4 substrate	CYP2B6 inhibitor	CYP2B6 substrate	CYP2C8 inhibitor	HLM Stability
01.	Beta-sitosterol	0.0	0.0	0.0	0.039	0.312	0.0	0.008	0.021	0.062	0.993	1.0	0.0	1.0	0.885
02.	Charantin	0.0	0.0	0.0	0.975	0.048	0.0	0.0	0.0	0.012	1.0	1.0	0.0	1.0	0.866
03.	Embelin	0.986	0.296	0.444	0.0	0.0	0.973	0.999	0.287	0.0	0.0	0.972	0.044	0.529	0.134
04.	Eugenol	0.997	0.334	0.993	0.991	0.601	1.0	0.342	0.999	0.998	0.0	0.999	0.043	1.0	0.077

05.	Amp-B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.499	0.0

Beta-sitosterol has a high probability of Human Liver Microsomal (HLM) instability (0.885), correlating with its strong substrate activity for CYP3A4 (0.993). Charantin It is highly unstable in human liver microsomes (0.963 probability of instability) and acts as a major CYP3A4 substrate (0.998). Embelin strongly inhibits several Cytochrome P450 enzymes, especially CYP1A2 (0.986), CYP2D6 (0.999), and CYP2B6 (0.972), posing a significant risk for drug-drug interactions. Eugenol is a potent inhibitor of CYP1A2 (0.97) and CYP2B6 (0.87). Amphotericin B is highly stable in human liver microsomes (0.0 probability of instability) (Table 08) [32], [33], [43].

3.2.4 Excretion

Table no. 09: Excretion analysis by using ADMET Lab 3.0

S. No.	Ligands	Excretion Factors	
		CL_{plasma}	$T_{1/2}$
01.	Beta-sitosterol	13.205	0.541
02.	Charantin	3.879	1.191
03.	Embelin	2.391	1.178
04.	Eugenol	10.004	1.464
05.	Amp - B	1.96	3.982

Beta-sitosterol and charantin have ultra-short half-lives of 0.541 hours and 0.655 hours, respectively. Embelin is categorized as a short half-life drug with a T1/2 of 1.178 hours and low plasma clearance of 2.391 ml/min/kg. Eugenol has a very short half-life of 0.672 hours, indicating rapid onset but limited duration of action. Amphotericin B exhibits low clearance (1.96 ml/min/kg) and a short half-life of approximately 3.98 hours (Table 09) [32], [33], [43].

3.2.5 Toxicity

Table no. 10: Toxicity analysis by using ADMET Lab 3.0

S. No.	Ligands	Toxicity Factors																				
		hERG Blocker	hERG Blocker (10u	DILI	AMES Mutagenici	Rat oral Acute Tox	FDAMDD	Skin Sensitizati	Carcinogenicity	Eye Corrosion	Eye Irritation	Respiratory	Human Hepatotoxi	Drug-induced Nep	Cytotoxicity	Hematotoxicity	Genotoxicity	RPMI-8226 immuni	A549 Cytotoxici	Hek293 Cytotoxic	Drug-induced Neu
01.	Beta-sitosterol	0.092	0.659	0.28	0.468	0.346	0.288	0.93	0.582	0.976	0.993	0.822	0.31	0.138	0.138	0.136	0.113	0.053	0.161	0.275	0.563
02.	Charantin	0.134	0.269	0.764	0.623	0.035	0.077	1.0	0.333	0.001	0.123	0.316	0.577	0.548	0.932	0.47	0.002	0.095	0.907	0.683	0.014
03.	Embelin	0.095	0.266	0.446	0.159	0.454	0.368	0.949	0.273	0.971	0.999	0.96	0.551	0.204	0.187	0.357	0.002	0.047	0.114	0.113	0.036
04.	Eugenol	0.092	0.659	0.28	0.468	0.346	0.288	0.93	0.582	0.976	0.993	0.822	0.31	0.138	0.138	0.136	0.113	0.053	0.161	0.275	0.563
05.	Amp-B	0.001	0.0	0.534	0.561	0.0	0.112	1.0	0.002	0.0	0.042	0.002	0.611	1.0	1.0	0.174	0.031	0.634	0.846	0.036	0.0

Table no. 11: Toxicophore analysis by using ADMET Lab 3.0

S.N	Liga	Toxicophore Factors							
		Acu	Genoto	Non-Ge	Skin	Aqu	Non-	Sur	FAF

o.	nd	teToxicityRule	xicity Carcinogenicity Rule	notoxicity Carcinogenicity Rule	Sensitization Rule	aticToxicityRule	Biodegradable Rule	eChEMBRule	-Drugs4Rule
01.	Beta-sitosterol	0	0	0	0	1	0	0	0
02.	Charantin	0	0	0	1	2	1	0	0
03.	Embelin	0	2	2	7	3	1	0	2 alerts
04.	Eugenol	0	0	0	5	0	0	0	2 alerts
05.	Amp-B	0	0	0	2	1	1	1	0

The Beta-sitosterol - It receives a score of 1.0, indicating it has a high *logP* (>3) and low *TPSA* (<75), a combination often associated with off-target toxicity. The probability scores are high for Skin Sensitization (0.99), Eye Irritation (0.956), and Respiratory Toxicity (0.873). On a positive note, the probabilities for Ames Mutagenicity (0.139) and Rat Oral Acute Toxicity (0.111) are low, suggesting a lack of immediate systemic lethality (Table 10 & 11) [32], [33], [43].

Charantin - The probability of Drug-Induced Liver Injury (0.278) and Nephrotoxicity (0.16) is very low. This is a significant advantage over Amphotericin B, which scored a 1.0 for nephrotoxicity. It shows a 0.0 probability of being an hERG blocker, suggesting a lack of cardiotoxicity risk. It is predicted to be non-mutagenic (Ames: 0.088) and non-carcinogenic (0.015) (Table 10 & 11) [32], [33], [43].

Embelin - It shows high probabilities for Skin Sensitization (0.949), Eye Irritation (0.999), and Respiratory Toxicity (0.96). Embelin shows high activity in stress-response pathways, notably the Antioxidant Response Element (SR-ARE: 0.985) and the p53 tumor suppressor protein pathway (SR-p53: 0.995). The high p53 probability often indicates DNA damage or cellular stress (Table 10 & 11) [32], [33], [43].

Eugenol shows a high probability of being a sensitizing agent with a value of 0.963. Other endpoints score even higher, for example, eye irritation and corrosion with 0.999 and 0.99 respectively. Regarding systemic endpoints, Eugenol scores low in most endpoints, e.g. mutagenicity and carcinogenicity with values of 0.016 and 0.038 respectively, making it a safe candidate from these endpoints. Further, neurotoxicity shows a very low score of 0.008. For nephrotoxicity, Eugenol does not score, indicating no concern (Table 10 & 11) [32], [33], [43].

Amp – B - Notably, the data confirms its known clinical profile with high probabilities for nephrotoxicity (1.0), ototoxicity (1.0), and skin sensitization (1.0). It also demonstrates significant A549 cytotoxicity (0.846) (Table 10 & 11) [32], [33], [43].

4 Conclusions

In conclusion, this investigation provides a robust pharmacological rationale for transitioning from conventional macrolide-based therapy to phytoconstituent-based nanomedicine for leishmaniasis. Molecular docking across seven regulatory receptors (1AI9, 6MJO, 7JUB, 4O2Z, 6WBG, 1E92, and 1E7W) characterized Beta-sitosterol as a high-affinity, multi-targeted lead, demonstrating a superior Re-rank score of -94.1387 against Sterol 14-alpha demethylase (1E7W). The binding in such interactions is stabilized with large packing of intermolecular hydrophobic contacts with such residues as Tyr116, Phe228, unlike the discrete anchoring observed for a smaller molecule such as Eugenol, or the dual site anchoring for Charantin (Table 05). Most significantly however, while conventional drug, Amphotericin B, has a

relatively high probability of producing nephrotoxicity (1.0), both Beta-sitosterol and Charantin provide a considerably greater toxicity profile (0.46 and 0.16, respectively) along with an increased drug-like profile. Extreme lipophilicity (LogP: 8.004) and the rapid CYP3A4 mediate liver turnover rate (T1/2: 0.54 h) for Beta-sitosterol are presented as the key pharmacokinetic limitations in ADMET profiling (Table 10 & 11) [32], [33], [43], [62].

This outcome makes the design of Nanostructured Lipid Carrier (NLC) essential for the clinical application. The NLC formulation overcome these issues rationally through pre-solubilizing the lipophilic backbone, enabling lymphatic system to reach Peyer's patch in order to evade first-pass effect and delivery to macrophages of spleen and liver specifically [27], [63], [64], [65], [66], [67].

This technique falls in line with today's nanotherapeutics approach where there is a focus on minimization of organ accumulation along with maximizing local ligand concentration. Further work needs to be done in order to verify IC50 values in vitro in regard to intracellular amastigotes as well as optimizing NLCs' in vivo release kinetics for increased biological residence time. Finally, the Beta-sitosterol-NLC drug formulation is one that is based upon scientific principles, lacks nephrotoxicity, and has oral bioavailability – all of which are improvements upon existing leishmaniasis therapy.

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ABBREVIATION: Visceral Leishmaniasis - VL, Lipid-based delivery systems – LBDS, Nanostructured Lipid Carrier – NLC, Dihydrofolate reductase - DHFR, Amphotericin – B – Amp – B, Fc gamma receptor - FGR, Mannose receptor – MMR, Mitogen Activated Protein Kinase – MPKs, Pannexin - PAN, Pteridine reductase - PTR, Protein Data Bank – PDB, Target Receptors – TR, Absorption, Distribution, Metabolism, Excretion & Toxicity – ADMET, Molegro Virtual Docker – MVD, Root Mean Square Deviation – RMSD.

5 References

[1] S. Mann *et al.*, "A Review of Leishmaniasis: Current Knowledge and Future Directions," *Curr. Trop. Med. Rep.*, vol. 8, no. 2, pp. 121–132, Mar. 2021, doi: 10.1007/s40475-021-00232-7.

[2] F. Divenuto, G. Pavia, N. Marascio, G. S. Barreca, A. Quirino, and G. Matera, "Role of Treg, Breg and other cytokine sets in host protection and immunopathology during human leishmaniasis: Are they potential valuable markers in clinical settings and vaccine evaluation," *Acta Trop.*, vol. 240, p. 106849, Apr. 2023, doi: 10.1016/j.actatropica.2023.106849.

[3] N. Singh, M. Kumar, and R. K. Singh, "Asian Pacific Journal of Tropical Medicine," 2012. [Online]. Available: www.elsevier.com/locate/apjtm.

[4] P. Prasanna *et al.*, "Current status of nanoscale drug delivery and the future of nano-vaccine development for leishmaniasis – A review," *Biomedicine & Pharmacotherapy*, vol. 141, p. 111920, Sep. 2021, doi: 10.1016/j.biopha.2021.111920.

[5] B. A. Khan *et al.*, "Fabrication and Evaluation of W/O Emulsion Loaded with Linum usitatissimum Seeds Extract for Anti-Leishmaniasis Efficacy," *Antibiotics*, vol. 11, no. 4, Apr. 2022, doi: 10.3390/antibiotics11040432.

[6] J. Martín-Sánchez, N. Torres-Medina, F. Morillas-Márquez, V. Corpas-López, and V. Díaz-Sáez, "Role of wild rabbits as reservoirs of leishmaniasis in a non-epidemic Mediterranean hot spot in Spain," *Acta Trop.*, vol. 222, p. 106036, Oct. 2021, doi: 10.1016/j.actatropica.2021.106036.

[7] R. M. T. E. Medeiros *et al.*, "Mapping linear B-cell epitopes of the Tryparedoxin Peroxidase and its implications in the serological diagnosis of tegumentary leishmaniasis," *Acta Trop.*, vol. 232, Aug. 2022, doi: 10.1016/j.actatropica.2022.106521.

[8] W. S. Mota *et al.*, "Isopentyl caffeate as a promising drug for the treatment of leishmaniasis: An in silico and in vivo study," *Curr. Res. Biotechnol.*, vol. 7, p. 100209, Jan. 2024, doi: 10.1016/j.crbiot.2024.100209.

[9] E. Gomes *et al.*, "Dataset of dual RNA-seq mapping in visceral leishmaniasis: Inquiry on parasite transcripts in human blood transcriptome upon Leishmania infantum infection," *Data Brief*, vol. 46, p. 108811, Feb. 2023, doi: 10.1016/j.dib.2022.108811.

[10] L. W. F. Souza, S. V. T. Souza, and A. C. C. Botelho, "Comparative analysis of the geographic distribution of the histopathological spectrum and Leishmania species of American cutaneous leishmaniasis in Brazil," *An. Bras. Dermatol.*, vol. 87, no. 3, pp. 369–374, Jun. 2012, doi: 10.1590/S0365-05962012000300003.

[11] J. V. Veasey, R. A. Zampieri, R. F. Lellis, T. H. P. de Freitas, and L. M. F. Winter,

"Identification of Leishmania species by high-resolution DNA dissociation in cases of American cutaneous leishmaniasis," *An. Bras. Dermatol.*, vol. 95, no. 4, pp. 459–468, Jul. 2020, doi: 10.1016/j.abd.2020.02.003.

[12] M. Bamorovat *et al.*, "Poor adherence is a major barrier to the proper treatment of cutaneous leishmaniasis: A case-control field assessment in Iran," *Int. J. Parasitol. Drugs Drug Resist.*, vol. 21, pp. 21–27, Apr. 2023, doi: 10.1016/j.ijpddr.2022.11.006.

[13] B. M. Roatt, J. M. de Oliveira Cardoso, R. C. F. De Brito, W. Coura-Vital, R. D. de Oliveira Aguiar-Soares, and A. B. Reis, "Recent advances and new strategies on leishmaniasis treatment," *Appl. Microbiol. Biotechnol.*, vol. 104, no. 21, pp. 8965–8977, Nov. 2020, doi: 10.1007/s00253-020-10856-w.

[14] A. G. España, M. I. F. Pimentel, J. P. de M. Lyra, C. M. Valete-Rosalino, and M. R. Lyra, "Description of the dermatoscopic features observed in sporotrichosis and American cutaneous leishmaniasis in a reference center in Rio de Janeiro, Brazil," *An. Bras. Dermatol.*, vol. 98, no. 6, pp. 764–773, Nov. 2023, doi: 10.1016/j.abd.2022.09.015.

[15] P. M. Kaye, I. Cruz, A. Picado, K. Van Bocxlaer, and S. L. Croft, "Leishmaniasis immunopathology—impact on design and use of vaccines, diagnostics and drugs," *Semin. Immunopathol.*, vol. 42, no. 3, pp. 247–264, Jun. 2020, doi: 10.1007/s00281-020-00788-y.

[16] P. Kropf *et al.*, "Toll-Like Receptor 4 Contributes to Efficient Control of Infection with the Protozoan Parasite *Leishmania major*," *Infect. Immun.*, vol. 72, no. 4, pp. 1920–1928, Apr. 2004, doi: 10.1128/IAI.72.4.1920-1928.2004.

[17] M. J. de Veer *et al.*, "MyD88 is essential for clearance of *Leishmania major* : possible role for lipophosphoglycan and Toll-like receptor 2 signaling," *Eur. J. Immunol.*, vol. 33, no. 10, pp. 2822–2831, Oct. 2003, doi: 10.1002/eji.200324128.

[18] P.-H. Tsai *et al.*, "Molecular diagnosis and therapy for cutaneous leishmaniasis of a returned traveler from Mexico," *Journal of Microbiology, Immunology and Infection*, vol. 54, no. 6, pp. 1154–1158, Dec. 2021, doi: 10.1016/j.jmii.2020.07.007.

[19] H. S. Sarwar *et al.*, "Redox Biology of *Leishmania* and Macrophage Targeted Nanoparticles for Therapy," *Nanomedicine*, vol. 12, no. 14, pp. 1713–1725, Jul. 2017, doi: 10.2217/nnm-2017-0049.

[20] M. Chaves, L. E. Savio, and R. Coutinho-Silva, "Purinergic signaling: A new front-line determinant of resistance and susceptibility in leishmaniasis," *Biomed. J.*, vol. 45, no. 1, pp. 109–117, Feb. 2022, doi: 10.1016/j.bj.2021.06.003.

[21] N. Baltaci, N. Aydogdu, C. Sarikurkcu, and B. Tepe, "Onosma gracilis (Trautv.) and O. oreodoxa (Boiss. & Heldr.): Phytochemistry, in silico docking, antioxidant and enzyme inhibitory activities," *South African Journal of Botany*, vol. 143, pp. 410–417, Dec. 2021, doi: 10.1016/j.sajb.2021.03.022.

[22] S. Thakur, J. Joshi, and S. Kaur, "Leishmaniasis diagnosis: an update on the use of parasitological, immunological and molecular methods," *Journal of Parasitic Diseases*, vol. 44, no. 2, pp. 253–272, Jun. 2020, doi: 10.1007/s12639-020-01212-w.

[23] K. Singh, G. Garg, and V. Ali, "Current Therapeutics, Their Problems and Thiol Metabolism as Potential Drug Targets in Leishmaniasis," *Curr. Drug Metab.*, vol. 17, no. 9, pp. 897–919, Aug. 2016, doi: 10.2174/1389200217666160819161444.

[24] R. Bansal, S. S. Sen, R. Muthuswami, and R. Madhubala, "Stigmasterol as a potential biomarker for amphotericin B resistance in Leishmania donovani," *Journal of Antimicrobial Chemotherapy*, vol. 75, no. 4, pp. 942–950, Apr. 2020, doi: 10.1093/jac/dkz515.

[25] A. Sidana and U. Farooq, "Evaluation of antileishmanial activity of plants used in Indian traditional medicine," *Bangladesh J. Pharmacol.*, vol. 10, no. 2, pp. 423–426, 2015, doi: 10.3329/bjp.v10i2.22674.

[26] M. Andima, G. Costabile, L. Isert, A. J. Ndakala, S. Derese, and O. M. Merkel, "Evaluation of β-sitosterol loaded PLGA and PEG-PLA nanoparticles for effective treatment of breast cancer: Preparation, physicochemical characterization, and antitumor activity," *Pharmaceutics*, vol. 10, no. 4, Dec. 2018, doi: 10.3390/pharmaceutics10040232.

[27] Y. Soleimanian *et al.*, "β-Sitosterol loaded nanostructured lipid carrier: Physical and oxidative stability, in vitro simulated digestion and hypocholesterolemic activity," *Pharmaceutics*, vol. 12, no. 4, Apr. 2020, doi: 10.3390/pharmaceutics12040386.

[28] K. Raj R, D. Ezhilarasan, and S. Rajeshkumar, "β-Sitosterol-assisted silver nanoparticles activates Nrf2 and triggers mitochondrial apoptosis via oxidative stress in human hepatocellular cancer cell line," *J. Biomed. Mater. Res. A*, vol. 108, no. 9, pp. 1899–1908, Sep. 2020, doi: 10.1002/jbm.a.36953.

[29] S. Majid Shah *et al.*, "β-Sitosterol from Ifloga spicata (Forssk.) Sch. Bip. as potential anti-leishmanial agent against leishmania tropica: Docking and molecular insights," *Steroids*, vol. 148, pp. 56–62, Aug. 2019, doi: 10.1016/j.steroids.2019.05.001.

[30] S. Singh, S. Vijaya Prabhu, V. Suryanarayanan, R. Bhardwaj, S. K. Singh, and V. K. Dubey, "Molecular docking and structure-based virtual screening studies of potential drug target, CAAX prenyl proteases, of *Leishmania donovani*," *J. Biomol. Struct. Dyn.*, vol. 34, no. 11, pp. 2367–

2386, Nov. 2016, doi: 10.1080/07391102.2015.1116411.
[31] B. Chandrasekaran, S. N. Abed, O. Al-Attraqchi, K. Kuche, and R. K. Tekade, "Computer-Aided Prediction of Pharmacokinetic (ADMET) Properties," in *Dosage Form Design Parameters*, Elsevier, 2018, pp. 731–755. doi: 10.1016/B978-0-12-814421-3.00021-X.
[32] E. Jamrozik, M. Śmieja, and S. Podlewska, "ADMET-PrInt: Evaluation of ADMET Properties: Prediction and Interpretation," *J. Chem. Inf. Model.*, vol. 64, no. 5, pp. 1425–1432, Mar. 2024, doi: 10.1021/acs.jcim.3c02038.
[33] E. L. Cáceres, M. Tudor, and A. C. Cheng, "Deep Learning Approaches in Predicting ADMET Properties," *Future Med. Chem.*, vol. 12, no. 22, pp. 1995–1999, Nov. 2020, doi: 10.4155/fmc-2020-0259.
[34] "CANDIDA ALBICANS DIHYDROFOLATE REDUCTASE," Nov. 12, 1997. doi: 10.2210/pdb1ai9/pdb.
[35] V. Van, W. D. Tolbert, and M. Pazgier, "CRYSTAL STRUCTURE OF RHESUS MACAQUE (MACACA MULATTA) FC-GAMMA RECEPTOR III," Nov. 13, 2019. doi: 10.2210/pdb6mjo/pdb.
[36] W. I. Weis and H. Feinberg, "C-type carbohydrate-recognition domain 4 of the mannose receptor complexed with methyl-mannoside," Feb. 03, 2021. doi: 10.2210/pdb7jub/pdb.
[37] A. K. Wernimont *et al.*, "Crystal Structure of MPK3 from Leishmania donovani, LdBPK_100540 in the presence of NVP-BBT594," Jan. 22, 2014. doi: 10.2210/pdb4o2z/pdb.
[38] "Cryo-EM structure of human Pannexin 1 channel with its C-terminal tail cleaved by caspase-7," Jun. 03, 2020. doi: 10.2210/pdb6wbg/pdb.
[39] D. G. Gourley and W. N. Hunter, "One active site, two modes of reduction correlate the mechanism of leishmania pteridine reductase with pterin metabolism and antifolate drug resistance in trpanosomes," Sep. 06, 2001. doi: 10.2210/pdb1e7w/pdb.
[40] A. W. Schuettelkopf and W. N. Hunter, "Pteridine reductase 1 from Leishmania major complexed with NADP+ and dihydrobiopterin," Oct. 05, 2001. doi: 10.2210/pdb1e92/pdb.
[41] P. Kaushik, S. Lal Khokra, A. C. Rana, and D. Kaushik, "Pharmacophore Modeling and Molecular Docking Studies on *Pinus roxburghii* as a Target for Diabetes Mellitus," *Adv. Bioinformatics*, vol. 2014, pp. 1–8, Jul. 2014, doi: 10.1155/2014/903246.
[42] H. A. Hemeg *et al.*, "In-Vitro Leishmanicidal Activity and Molecular Docking Simulations of a Flavonoid Isolated from Pistacia integerrima Stew ex Brandis," *J. Food Qual.*, vol. 2022, 2022, doi: 10.1155/2022/6003869.
[43] S. Maamri, K. Benarous, and M. Yousfi, "Identification of 3-Methoxycarpachromene and Masticadienonic Acid as New Target Inhibitors against Trypanothione Reductase from Leishmania Infantum Using Molecular Docking and ADMET Prediction," *Molecules*, vol. 26, no. 11, p. 3335, Jun. 2021, doi: 10.3390/molecules26113335.
[44] A. N. Tevyashova *et al.*, "Structure-Antifungal Activity Relationships of Polyene Antibiotics of the Amphotericin B Group," *Antimicrob. Agents Chemother.*, vol. 57, no. 8, pp. 3815–3822, Aug. 2013, doi: 10.1128/AAC.00270-13.
[45] H. Ma *et al.*, "Design, Synthesis, and Structure–Activity Relationship Studies of Bisamide Derivatives of Amphotericin B with Potent Efficacy and Low Toxicity," *J. Med. Chem.*, vol. 65, no. 13, pp. 8897–8913, Jul. 2022, doi: 10.1021/acs.jmedchem.1c02227.
[46] P. Kumar Singh *et al.*, "Macrophage targeted amphotericin B nanodelivery systems against visceral leishmaniasis," *Materials Science and Engineering: B*, vol. 258, p. 114571, Aug. 2020, doi: 10.1016/j.mseb.2020.114571.
[47] H. Ma *et al.*, "Design, Synthesis, and Structure–Activity Relationship Studies of Bisamide Derivatives of Amphotericin B with Potent Efficacy and Low Toxicity," *J. Med. Chem.*, vol. 65, no. 13, pp. 8897–8913, Jul. 2022, doi: 10.1021/acs.jmedchem.1c02227.
[48] L. Ravi, S. Girish, M. Harshini, and B. K. A. Sreenivas, "β-Sitosterol: An Antibacterial Agent in Aquaculture Management of Vibrio Infections," *J. Pure Appl. Microbiol.*, vol. 14, no. 4, pp. 2699–2714, Dec. 2020, doi: 10.22207/JPAM.14.4.48.
[49] J. Dwivedi, P. Sachan, and P. Wal, "A Mechanistic Approach on Structural, Analytical and Pharmacological Potential of Beta-sitosterol: A Promising Nutraceutical," *Curr. Nutr. Food Sci.*, vol. 20, no. 8, pp. 932–951, Oct. 2024, doi: 10.2174/0115734013245468230927042947.
[50] A. A. Tayel *et al.*, "Construction of bioactive nanocomposites from beta-sitosterol, fucoidan, chitosan, and silver nanoparticles for anticancer applications," *Front. Bioeng. Biotechnol.*, vol. 13, Oct. 2025, doi: 10.3389/fbioe.2025.1668888.
[51] C. Nweze, H. Ibrahim, and G. I. Ndukwe, "Beta-sitosterol with antimicrobial property from the stem bark of pomegranate (<i>Punica granatum</i> Linn)," *Journal of Applied Sciences and Environmental Management*, vol. 23, no. 6, p. 1045, Jul. 2019, doi: 10.4314/jasem.v23i6.7.
[52] S. Desai, P. Tatke, T. Mane, and S. Gabhe, "Isolation, characterization and quantitative HPLC-DAD analysis of components of charantin from fruits of Momordica charantia," *Food Chem.*, vol. 345, p. 128717, May 2021, doi: 10.1016/j.foodchem.2020.128717.
[53] G. Abbas *et al.*, "Charantin targets HMGCR-PCSK9 axis and activates PPAR-α

signaling to ameliorate hyperlipidemia: Mechanistic insights from bioinformatics and in-vivo studies," *PLoS One*, vol. 20, no. 9, p. e0331356, Sep. 2025, doi: 10.1371/journal.pone.0331356.
[54] N. J. Basha, S. M. Basavarajaiah, S. Baskaran, and P. Kumar, "A comprehensive insight on the biological potential of embelin and its derivatives," *Nat. Prod. Res.*, vol. 36, no. 12, pp. 3054–3068, Jun. 2022, doi: 10.1080/14786419.2021.1955361.
[55] Z. Sheng *et al.*, "Synthesis and Biological Activity of Embelin and its Derivatives: An Overview," *Mini-Reviews in Medicinal Chemistry*, vol. 20, no. 5, pp. 396–407, Apr. 2020, doi: 10.2174/1389557519666191015202723.
[56] Z. Sheng *et al.*, "Synthesis and Biological Activity of Embelin and its Derivatives: An Overview," *Mini-Reviews in Medicinal Chemistry*, vol. 20, no. 5, pp. 396–407, Apr. 2020, doi: 10.2174/1389557519666191015202723.
[57] K. Triatmaja, S. Prabawati, and P. Widiakongko, "QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR) STUDY OF EUGENOL DERIVATIVES AS ANTIOXIDANT COMPOUNDS," in *Proceedings of the International Conference on Food, Nutrition, Health and Lifestyle*, The International Institute of Knowledge Management, Oct. 2022, pp. 10–18. doi: 10.17501/26827026.2022.1102.
[58] R. R. Teixeira *et al.*, "Eugenol derivatives with 1,2,3-triazole moieties: Oral treatment of cutaneous leishmaniasis and a quantitative structure-activity relationship model for their leishmanicidal activity," *Exp. Parasitol.*, vol. 238, p. 108269, Jul. 2022, doi: 10.1016/j.exppara.2022.108269.
[59] S. K. Venkatesan, A. K. Shukla, and V. K. Dubey, "Molecular docking studies of selected tricyclic and quinone derivatives on trypanothione reductase of *Leishmania infantum*," *J. Comput. Chem.*, vol. 31, no. 13, pp. 2463–2475, Oct. 2010, doi: 10.1002/jcc.21538.
[60] N. E. Thomford *et al.*, "Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery," *Int. J. Mol. Sci.*, vol. 19, no. 6, p. 1578, May 2018, doi: 10.3390/ijms19061578.
[61] C. Al Khoury, G. Nemer, J. Guillot, and S. Tokajian, "Absolute quantification of gene expression in drug discovery using RT-qPCR: Case of a drug used in the treatment of leishmaniasis," *Res. Vet. Sci.*, vol. 153, pp. 17–22, Dec. 2022, doi: 10.1016/j.rvsc.2022.10.012.
[62] L. Raju, R. Lipin, and R. Eswaran, "Identification, ADMET evaluation and molecular docking analysis of Phytosterols from Banaba (Lagerstroemia speciosa (L.)Pers) seed extract against breast cancer," *In Silico Pharmacol.*, vol. 9, no. 1, p. 43, Jul. 2021, doi: 10.1007/s40203-021-00104-y.
[63] T. V. Jagtap, A. R. Madgulkar, and M. R. Bhalekar, "Biochemical investigations, in silico study and targeted delivery aspects of plant phytosterols: β-sitosterol," *Inflammopharmacology*, vol. 33, no. 11, pp. 6329–6355, Nov. 2025, doi: 10.1007/s10787-025-01951-3.
[64] M. R. Aji Alex, A. J. Chacko, S. Jose, and E. B. Souto, "Lopinavir loaded solid lipid nanoparticles (SLN) for intestinal lymphatic targeting," *European Journal of Pharmaceutical Sciences*, vol. 42, no. 1–2, pp. 11–18, Jan. 2011, doi: 10.1016/j.ejps.2010.10.002.
[65] J. F. Fangueiro *et al.*, "A novel lipid nanocarrier for insulin delivery: production, characterization and toxicity testing," *Pharm. Dev. Technol.*, vol. 18, no. 3, pp. 545–549, Jun. 2013, doi: 10.3109/10837450.2011.591804.
[66] S. M. M. Moghddam, A. Ahad, M. Aqil, S. S. Imam, and Y. Sultana, "Optimization of nanostructured lipid carriers for topical delivery of nimesulide using Box–Behnken design approach," *Artif. Cells Nanomed. Biotechnol.*, vol. 45, no. 3, pp. 617–624, Apr. 2017, doi: 10.3109/21691401.2016.1167699.
[67] G. Poovi and N. Damodharan, "Lipid nanoparticles: A challenging approach for oral delivery of BCS Class-II drugs," *Futur. J. Pharm. Sci.*, vol. 4, no. 2, pp. 191–205, Dec. 2018, doi: 10.1016/j.fjps.2018.04.001.

Figures:

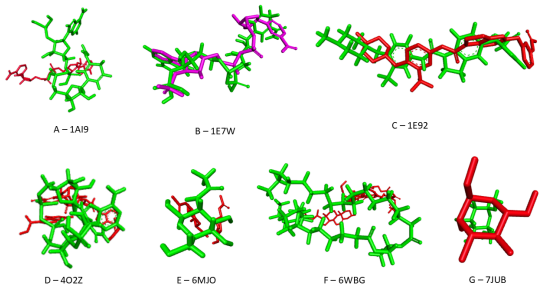


Fig No. 1: RMSD validation of various target receptors

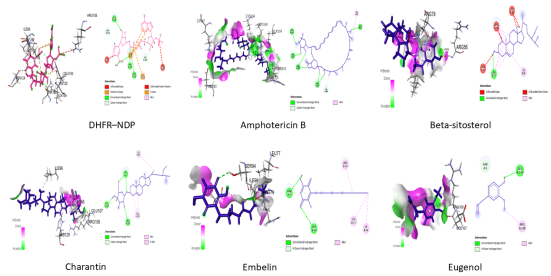


Fig No. 2: 2D & 3D structure of interaction between the receptor (1AI9) with all selected ligands.

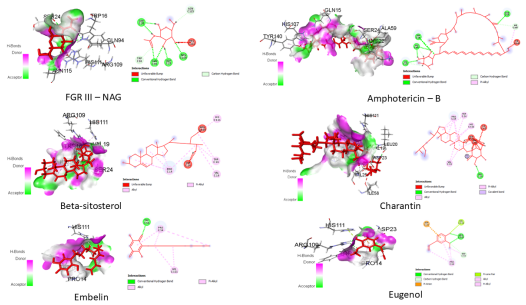


Fig No. 03: 2D & 3D structure of interaction between the receptor (6MJO) with all selected ligands.

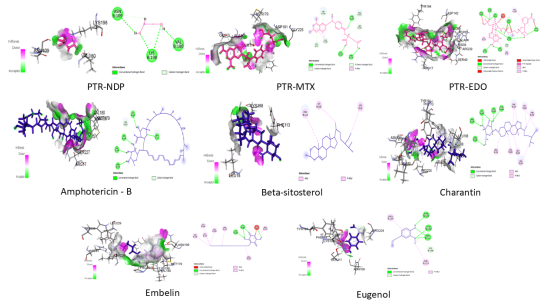


Fig No.04: 2D & 3D structure of interaction between the receptor (1E7W) with all selected ligands.

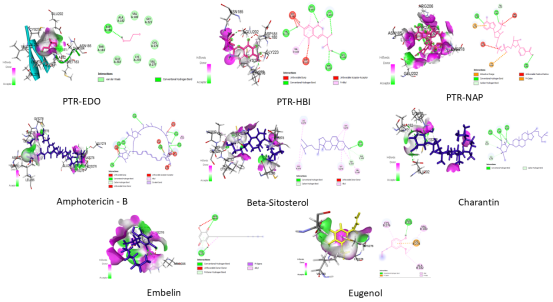


Fig No. 05: 2D & 3D structure of interaction between the receptor (1E92) with all selected ligands.

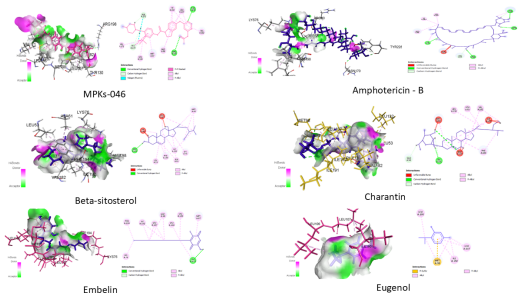


Fig No. 06: 2D & 3D structure of interaction between the receptor (4O2Z) with all selected ligands.

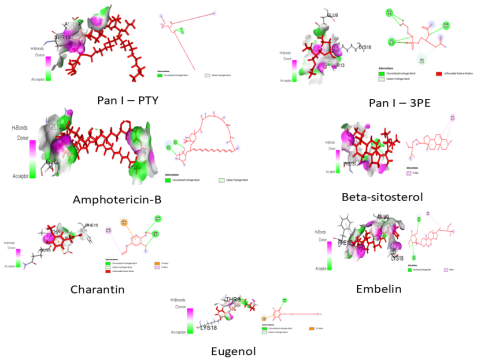


Fig No. 07: 2D & 3D structure of interaction between the receptor (6WBG) with all selected ligands.

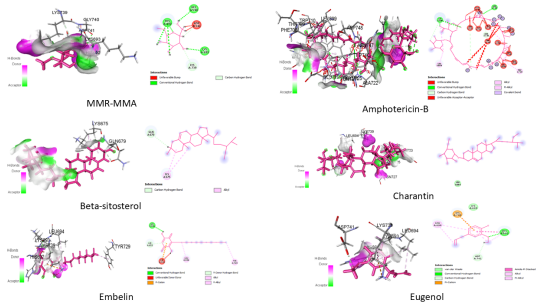


Fig No. 08: 2D & 3D structure of interaction between the receptor (7JUB) with all selected ligands.

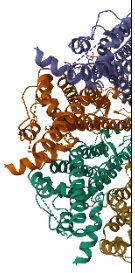
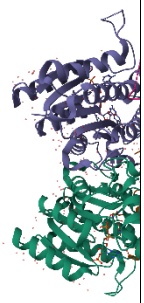
Tables:

Table no 01: List of selected receptors for Leishmaniasis

S. No.	Receptors	PDB ID	Image	Mechanism of Action	References
01.	Dihydrofolate reductase (DHFR)	1AI9		DHFR catalyzes the reduction of dihydrofolate to trihydrofolate, ensuring a steady supply of this essential	[34]

				cofactor. This becomes the prime target for anti-parasitic drug development compounds, that inhibit DHFR can disrupt the parasite's metabolic pathways leading to death or impaired growth.	
02.	Fc gamma receptor – III (FGR-III)	6MJO		These receptors are expressed on the surface of various immune cells, including macrophages, neutrophils and dendritic cells and are responsible for the recognizing and binding to the Fc portion of antibodies. They activate the macrophage for phagocytosis.	[35]
03.	Mannose receptor (MMR)	7JUB		Mannose residues on the surface of the leishmanial parasite bind to the mannose receptors on the	[36]

				host macrophages.	
04.	Mitogen Activated Protein Kinase (MPKs)	4O2Z		MPKs are activated in response to Leishmanial infection, playing a role in both innate and adaptive immune responses. They can influence the production of cytokinase and chemokinase, which are essential for recruiting immune cells to infection site.	[37]

05.	Pannexin – I (PAN - I)	6WBG		The pannexin facilitates the release of ATP, which acts as a danger signal and recruits immune cells to the site of infection.	[38]
06.	Pteridine reductase (PTR - I)	1E92		PTR is essential for the leishmaniasis to produce the folate, which is vital for the their growth and replication.	[39], [40]

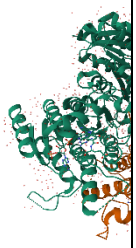
07.	Pteridine reductase (PTR - I)	1E7W		PTR is essential for the leishmaniasis to produce the folate, which is vital for the their growth and replication.	[39], [40]

Table no 02: List of selected ligands with 2D structure.

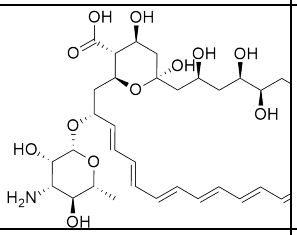
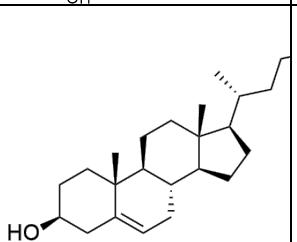
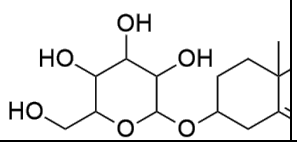
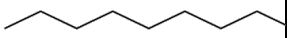
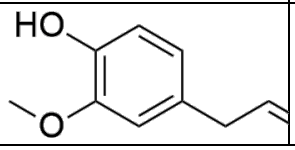
S. No.	Ligands	2D structure	References
01.	Amphotericin- B (Standard Ligand)		[24], [44], [45], [46], [47]
02.	Beta-sitosterol		[48], [49], [50], [51]
03.	Charantin		[52], [53]
04.	Embelin		[54], [55], [56]
05.	Eugenol		[57], [58]

Table no 03: Validation of Mol Dock Protocol

Molecules	Root Mean Square Deviation (RMSD) values in various receptors						
	1AI9	1E7W	1E92	4O2Z	6MJO	6WBG	7JUB
Amphotericin- B (Standard Ligand)	0.000	0.00	0.000	0.00	0.00	0.000	0.00
Beta-sitosterol	0.000	0.00	0.000	0.00	0.00	0.000	0.00
Charantin	0.000	0.00	0.000	0.00	0.00	0.000	0.00
Embelin	0.000	0.00	0.000	0.00	0.00	0.000	0.00
Eugenol	0.000	0.00	0.000	0.00	0.00	0.000	0.00

Table no. 04: Result of Molecular Docking between the various binding receptors and ligands.

S. No.	Receptors with PDB ID	Ligands	Pose Energy	Re-rank	H-bond	Amino Acids
01	1AI9	Beta - sitosterol	-98.9031	-12.0444	-2.5	Chain A: Arg 108, Asn 5, Glu 107, His 129, Phe 167, Pro 4.

						Chain B: Ala 93, Arg 79, Glu 97, Glu 116, Glu 120, Gly 55, Ile96, Ile117, Leu 77, Ser 78, Ser 94, Ser 95.
		Charantin	-110.793	-83.6748	-9.0652	Chain A: Arg 108, Arg 191, Asn 5, Glu 107, Glu 163, His 129, Lys 192, Phe 167, Pro 4, Ser 128, Thr 190. Chain B: Asn 123, Asn 124, Glu 97, Glu 120, Ile96, Ser 94, Ser 95, Ser 125.
		Embelin	-98.2568	-77.6327	-3.4921	Chain A: Arg 108, Asn 5, Glu 107, Lys 3, Met 1, Pro 4, Val 109. Chain B: Arg 79, Asn 83, Glu 97, Glu 120, Ile96, Ile117, Leu 77, Ser 78, Ser 94, Ser 95, Ser 98.
		Eugenol	-69.0524	-55.1374	-2.5	Chain A: Arg 108, Asn 5,

						Glu 107, Lys 3, Met 1, Pro 4, Val 109. Chain B: Asn 83, Ser 94, Ser 95, Ser 98.
		Amphotericin – B (Standard Drug)	-128.889	-118.749	-17.706	Chain A: Arg 108, Arg 191, Asn 5, Gln 159, Glu 107, Glu 163, His 129, Lys 45, Lys 166, Lys 192, Phe 167, Thr 44, Thr 157, Thr 190. Chain B: Arg 79, Asn 123, Asn

						124, Glu 82, Glu 97, Glu 120, Ser 94, Ser 95, Ser 125, Tyr 81.
		DHFR – NDP (Internal Standard)	-184.285	-66.5347	-3.7883	Chain A: Arg 191, Asn 5, Gln 159, Glu 163, His 129, Leu 188, Lys 166, Phe 167, Ser 128, Thr 190. Chain B: Asn 123, Asn 124, Glu 97, Glu 120, Ile96, Leu 121, Ser 94, Ser 95.

2	6MJO	Beta-sitosterol	-49.4348	57.1872	0.0	Chain C: Arg18, Arg109, Asn115, Asp23, Gln15, Gln94, Glu13, His111, Phe57, Pro14, Ser24, Thr26, Trp16, Val19, Val25.
		Charantin	121.334	1105.01	-1.1169	Chain C: Ala59, Ala60, Ala61, Arg18, Arg62, Arg109, Asn65, Asn115, Asp23, Gln94, Glu13, Glu21, Glu85, His111, Ile58, Leu20, Leu84, Lys22, Pro14, Ser24, Trp16, Val19, Val25, Val86.
		Embelin	-44.7863	-49.2979	-2.50	Chain C: Arg109, Asn115, Asp23, Gln94, Glu13, His111, Lys22, Pro14, Ser24, Trp16, Val25.
		Eugenol	-58.258	-37.7384	-7.89466	Chain C: Arg109, Asn115, Asp23, Gln94,

						His 111, Pro 14, Ser 24, Trp 16, Val 25.
		Amphotericin-B (Standard Drug)	9840.74	-82.7203	-9.4134	Chain C: Ala 59, Ala 60, Arg 109, Asn 115, Asp 23, Asp 138, Gln 15, Glu 13, His 107, Lys 22, Lys 114, Phe 57, Pro 14, Ser 24, Trp 16, Tyr 140, Val 25.
		FGR III – NAG (Internal Standard)	-56.6087	7.9746	-16.651	Chain C: Arg 18, Arg 109, Asn 115, Asp 23, Gln 94, Glu 13, His 111, Pro 14, Ser 24, Trp 16, Val 19, Val 25.
03	1E7W	Beta-sitosterol	9879.08	-94.1387	0.0	Chain B: Ala 182, Arg 17, Asn 109, Asp 181, Gly 225, Leu 18, Leu 226, Leu 229, Lys 198, Met 179, Phe 113, Pro 224, Ser 111, Ser 112, Ser 227, Tyr 194, Val 180, Val 228.
		Charantin	9841.54	-100.352	-8.3626	Chain B: Ala 110, Arg 17,

						Asn 109, Asn 147, Asp 181, Gln 186, Gly 225, His 241, Leu 18, Leu 188, Leu 226, Leu 229, Lys 198, Met 179, Met 183, Phe 113, Pro 187, Pro 224, Ser 111, Ser 227, Trp 238, Tyr 191, Tyr 194, Tyr 283, Val 180, Val 222.
		Embelin	-13.6674	-53.8156	-4.42226	Chain B: Ala 110, Ala 182, Asn 108, Asn 109, Asn 147, Asp 181, Gly 223, Gly 225, His 241, Leu 18, Leu 226, Leu 229, Lys 198, Met 179, Phe 113, Pro 224, Ser 111, Ser 227, Trp 238, Tyr 194, Val 180.
		Eugenol	-70.6319	-57.0959	-6.45088	Chain B: Ala 110, Asn 109, Asn 147, Asp 181, Gly 223, Gly 225, Leu 18, Leu 226, Lys 198, Met 179, Phe 113, Pro 224,

						Ser 111, Ser 112, Tyr 194, Val 180.
		Amphotericin-B (Standard Drug)	9806.32	-128.804	-9.3712	Chain B: Ala 110, Arg 17, Asn 109, Asn 147, Asp 181, Gly 225, Leu 18, Leu 226, Leu 229, Lys 198, Met 179, Phe 113, Pro 115, Pro 224, Ser 111, Ser 112, Ser 227, Trp 238, Tyr 114, Tyr 194, Val 180, Val 228.
		PTR-EDO (Internal	-41.1932	-29.2771	-7.1357	Chain B: Ala 110,
		Standard)				Asn 109, Asp 181, Lys 198, Met 179, Pro 224, Ser 111, Val 180.
		PTR-MTX (Internal Standard)	9851.59	-104.683	-3.7483	Chain B: Ala 110, Arg 17, Asn 109, Asp 181, Gly 13, Gly 225, His 36, His 38, Leu 18, Leu 226, Lys 198, Met 179, Phe 113, Pro 224, Ser 111, Ser 112, Ser 227, Thr 12, Tyr 37, Tyr 194, Val 180.

		PTR-NDP (Internal Standard)	9721.24	-135.717	-24.861	Chain B: Ala 14, Ala 15, Ala 64, Ala 110, Arg 17, Arg 39, Asn 109, Asn 147, Asp 65, Asp 142, Asp 181, Gly 13, Gly 223, Gly 225, His 36, His 38, Leu 18, Leu 66, Lys 16, Lys 198, Met 179, Phe 113, Pro 224, Ser 40, Ser 67, Ser 111, Ser 112, Ser 146, Thr 12, Tyr 37, Tyr 194, Val 180.
04	1E92	Beta-sitosterol	-114.941	-83.5803	-0.8022	Chain A: Ala 182, Arg 206, Asn 185, Asp 181, Cys 276, Glu 202, Gly 223, Lys 278, Val 180, Val 277. Chain C: Ala 182, Arg 206, Asn 185, Asp 181, Cys 276, Glu 202, Gly 223, Lys 278, Val 180, Val 222, Val 277. Chain D: Asn 185.

		Charantin	-128.671	-82.5419	-1.30855	Chain A: Ala 182, Arg 206, Asn 185, Asp 181, Cys 276, Glu 202, Gly 223, Lys 278, Thr 184, Val 180, Val 222, Val 277. Chain B: Asn 185, Lys 278. Chain C: Arg 206, Asn 185, Glu 202. Chain D: Arg 206, Asn 185, Glu 202.
		Embelin	-95.8891	-61.4626	-0.6647	Chain A: Ala 182, Arg 206,
						Asn 185, Asp 181, Cys 276, Glu 202, Gly 274, Lys 278, Thr 184, Thr 275, Val 180, Val 277. Chain B: Asn 185. Chain C: Cys 276, Lys 278.
		Eugenol	-68.9988	-53.5986	-2.5	Chain B: Ala 182, Arg 206, Asn 185, Asp 181, Cys 276, Glu 202, Gly 223, Lys 278, Pro 224, Thr 184, Val 180, Val 222, Val

						277, Val 279.
		Amphotericin – B (Standard Drug)	-86.0612	546.679	-17.028	Chain A: Ala 182, Arg 206, Asn 185, Asp 181, Cys 276, Glu 202, Gly 223, Lys 278, Thr 275, Val 180, Val 277. Chain B: Ala 182, Arg 206, Asn 185, Asp 280, Cys 276, Glu 202, Gly 282, Leu 285, Lys 278, Thr 275, Val 277, Val 279. Chain C: Asn 185,

						Cys 276, Gly 274, Lys 278, Thr 275, Val 277. Chain D: Arg 206, Cys 276, Gly 274, Ile272, Thr 273, Thr 275, Val 277.
		PTR-EDO (Internal Standard)	-43.8294	-35.3179	-2.5184	Chain A: Ala 182, Asp 181, Cys 276, Glu 202, Gly 223, Lys 278, Val 180, Val 222, Val 277.
		PTR-HBI (Internal Standard)	-102.163	-37.2042	-8.8096	Chain D: Ala 182, Arg 206, Asn 185, Asp

						181, Cys 276, Glu 202, Gly 221, Gly 223, Lys 278, Val 180, Val 222, Val 277, Val 279.
		PTR-NAP (Internal Standard)	-149.834	-67.6957	-9.7338	Chain A: Arg 206, Asn 185, Cys 276, Glu 202, Gly 274, Lys 278, Thr 275. Chain B: Arg 206, Asn 185, Glu 202, Lys 278. Chain C: Arg 206, Asn 185, Glu 202, Lys 278. Chain D: Arg 206, Asn 185, Asp 280, Glu 202, Lys 278.
05	4O2Z	Beta-sitosterol	-130.581	-10.6502	-2.5	Chain A: Ala 61, Arg 198, Asp 134, Asp 136, Asp 193, Glu 94, Ile191, Ile192, Leu 53, Leu 107, Leu 132, Lys 76, Met 97, Met 98, Met 133, Phe 194, Pro 131, Ser 74, Thr 130, Val 128, Val 135, Val 182.

		Charantin	-142.872	64.2792	-2.8630	Chain A: Ala 61, Ala 179, Arg 198, Asn 180, Asp 134, Asp 136, Asp 193, Glu 94, His 173, Ile1 91, Ile1 92, Leu 53, Leu 106, Leu 107, Leu 132, Leu 166, Leu 181, Lys 76, Met 97, Met 98, Met 133, Phe 101, Phe 194, Pro 131, Pro 178, Ser 74, Thr 130, Val 135,

						Val 182.
		Embelin	-126.702	-99.6767	-2.5	Chain A: Ala 172, Asp 175, Asp 193, Glu 94, His 173, Ile1 91, Ile1 92, Leu 106, Leu 107, Leu 166, Leu 196, Lys 76, Met 97, Met 98, Phe 101, Phe 194, Val 171.
		Eugenol	-77.5228	-61.9515	-2.5	Chain A: Asp 193, Glu 94, His 173, Ile1 91, Ile1 92, Leu 106, Leu 107, Leu 166, Met 97,

						Met 98, Phe 101, Phe 194.
		Amphotericin – B (Standard Drug)	-116.859	12.1729	-8.8988	Chain A: Ala 172, Arg 90, Arg 93, Arg 174, Asn 170, Asn 227, Asp 175, Asp 193, Glu 94, Glu 355, Gly 195, His 173, Ile1 92, Leu 107, Leu 196, Lys 76, Met 97, Met 98, Phe 194, Ser 229, Thr 130, Tyr 58, Tyr 228, Val 128, Val 171.

		MPKs-046 (Internal Standard)	-143.576	-106.734	-1.8761	Chain A: Ala 172, Ala 179, Arg 93, Arg 174, Arg 198, Asp 136, Asp 175, Asp 193, Glu 94, His 173, Ile1 91, Ile1 92, Leu 53, Leu 106, Leu 107, Leu 166, Lys 76, Met 97, Met 98, Met 133, Phe 101, Phe 194, Pro 131, Ser 74, Thr 130, Tyr 228, Val 171, Val 182.

06	6WBG	Beta-sitosterol	-88.9247	-64.5859	0.0	Chain A: Gln 5, Phe 12, Ser 13, Thr 8. Chain G: Asp 14, Gln 5, Glu 9, Leu 16, Lys 18, Phe 15, Ser 13, Thr 8, Tyr 10.
		Charantin	-109.908	-70.9045	-4.0742	Chain A: Asp 14, Gln 5, Glu 9, Leu 16, Lys 18, Phe 15, Ser 13, Thr 8 Tyr 10. Chain B: Phe 12, Ser 13, Thr 8. Chain G: Asp 14, Gln 5, Glu 9, Lys 18, Phe 15, Ser 13, Thr 8.
		Embelin	-81.1747	-60.9382	-2.5	Chain A: Asp 14, Gln 5, Glu 9, Leu 6, Phe 12, Ser 13, Thr 8. Chain B: Gln 5. Chain G: Leu 16, Lys 18, Phe 15.
		Eugenol	-58.6872	-42.8539	-3.4336	Chain A: Lys 18. Chain B: Asp 14,

						Gln 5, Glu 9, Lys 18, Phe 15, Ser 13, Thr 8, Tyr 10.
		Amphotericin – B (Standard Drug)	-125.8455	-67.4191	-8.4420	Chain A: Gln 5. Chain B: Glu 9. Chain C: Gln 5, Glu 9. Chain D: Gln 5. Chain F: Glu 9. Chain G: Gln 5, Glu 9, Leu 6, Thr 8.
		Pan I – 3PE (Internal Standard)	90.1801	20.0376	-2.5	Chain A: Glu 22, Lys 18, Thr 21. Chain B: Asp 14, Glu 9, Phe 12, Ser 13.
		Pan I – PTY (Internal Standard)	-74.3755	-47.4748	-6.6648	Chain B: Glu 9, Lys 18, Phe 15. Chain C: Gln 5, Glu 9, Phe 12, Phe 15, Ser 13, Thr 8.
07	7JUB	Beta - sitosterol	-1.7258	-28.2604	0	Chain A: Ala 722, Asn 720, Gln 678, Gln 679, Glu 676, Lys 675, Met 744, Pro 742, Ser 745, Thr 743,

						Trp 682, Trp 721, Tyr 723.
		Charantin	202.60	21.9841	0	Chain A: Glu 725, Leu 694, Tyr 723.
		Embelin	-78.4101	-58.9812	-2.9172	Chain A: Asn 727, Asn 747, Asp 741, Asp 748, Glu 725, Glu 733, Glu 737, Gly 740, His 692, Ile749, Leu 694, Lys 693, Lys 739, Tyr 691, Tyr 729.
		Eugenol	-66.9066	-47.8875	-2.4957	Chain A: Asp 741, Gly 740, His 692, Leu 694, Lys 693, Lys 739, Tyr 691.
		Amphotericin-B (Standard Drug)	675.33	4343.44	17.585	Chain A: Ala 722, Asn 720, Asn 747, Asp 748, Cys 735, Glu 719, Glu 725, Glu 737, Gly 698, Gly 707, Gly 724, Gly 736, His 692, Leu 694, Leu 697, Leu 699, Leu 738, Lys 693, Lys 739, Phe 708, Pro 726, Ser 745, Thr 700, Thr 709, Trp 710, Trp 721,

						Trp 746, Tyr 718, Tyr 723, Val 716.
		MMR-MMA (Internal Standard)	-67.0294	-23.7145	-3.7639	Chain A: Asp 741, Gly 740, His 692, Leu 694, Lys 693, Lys 739, Tyr 691.

Table no. 05: Summary of Mol Dock results of All Ligands with selected target receptors.

S.No.	Target Receptor (PDB ID)	Amphotericin B	Beta-sitosterol	Charantin	Embelin	Eugenol
01	DHFR (1AI9)	High (-101.4)	Moderate (-68.2)	Moderate (-71.5)	High (-84.1)	Low (-42.3)
02	FGR-III (6MJO)	High (-98.7)	High (-82.4)	Low (-54.1)	Moderate (-62.8)	Low (-38.5)
03	MMR (7JUB)	High (-95.2)	High (-79.1)	Moderate (-65.4)	Moderate (-59.3)	Low (-40.1)
04	MPKs (4O2Z)	High (-89.6)	Moderate (-62.1)	Moderate (-68.9)	High (-78.4)	Low (-4
						4.2)
05	PAN-I (6WBG)	Moderate (-68.4)	High (-51.3)	High (-78.1)	Moderate (-55.2)	High (-58.7)
06	PTR-I (1E92)	High (-105.3)	High (-91.5)	High (-80.2)	Moderate (-51.6)	Low (-41.8)
07	PTR-I (1E7W)	High (-112.4)	High (-94.1)	High (-82.5)	Moderate (-53.8)	Low (-43.6)

Table no. 06: Absorption analysis by using ADMET Lab 3.0

S.No.	Ligand	Absorption Factors								
		Caco-2 Permeability	MDCK Permeability	PAMPA	Pgp-inhibitor	Pgp-substrate	HIA	F20%	F30%	F50%
01	Beta-sitosterol	-5.122	-4.936	0.092	0.0	0.219	0.0	0.002	0.065	0.987
02	Charantin	-5.382	-5.103	0.426	0.0	0.03	0.0	0.001	0.334	0.994
03	Em	-5.	-4.	0.	0.	0.	0.	0.	0.	0.

	belin	023	774	281	017	044	985	789	998	995
04	Eugenol	-4.570	-4.652	0.004	0.468	0.002	0.035	0.954	0.985	0.971
05	Amp-B	-5.784	-5.329	1.0	0.0	1.0	0.013	0.992	1.0	1.0

Table no. 07: Distribution analysis by using ADMET Lab 3.0

S.No.	Ligand	Distribution Factors							
		PPB	VDss	BBB	Fu	OATP1B1 inhibitor	OATP1B3 inhibitor	BCRP inhibitor	MRP1 inhibitor
01.	Beta-sitosterol	86.939	-0.244	0.045	12.443	1.0	1.0	0.349	0.019
02.	Charantin	79.683	-0.473	0.021	16.191	1.0	1.0	0.145	0.001
03.	Embelin	97.372	0.345	0.0	2.368	0.968	1.0	0.69	0.943
04.	Eugenol	80.610	0.122	0.106	20.837	0.999	0.998	0.914	0.418
05.	Amp-B	63.6	-0.5	0.0	34.2	1.0	1.0	0.005	0.591

		11	03		68					

Table no. 08: Metabolism analysis by using ADMET Lab 3.0

S.No.	Ligands	Metabolism Factors												
		CYP1A2 Inhibitor	CYP1A2 Substrate	CYP2C19 inhibitor	CYP2C19 substrate	CYP2C9 inhibitor	CYP2C9 substrate	CYP2D6 inhibitor	CYP2D6 substrate	CYP3A4 inhibitor	CYP3A4 substrate	CYP2B6 inhibitor	CYP2B6 substrate	CYP2C8 inhibitor	HLM Stability
01.	Beta-sitosterol	0.0	0.0	0.0	0.039	0.312	0.0	0.008	0.021	0.062	0.993	1.0	0.0	1.0	0.885
02.	Charantin	0.0	0.0	0.0	0.975	0.048	0.0	0.0	0.0	0.012	1.0	1.0	0.0	1.0	0.866
03.	Embelin	0.986	0.296	0.444	0.0	0.0	0.973	0.999	0.287	0.0	0.0	0.972	0.044	0.529	0.134
04.	Eugenol	0.997	0.334	0.993	0.991	0.601	1.0	0.342	0.999	0.998	0.0	0.999	0.043	1.0	0.077

05.	Amp-B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.499	0.0

Table no. 09: Excretion analysis by using ADMET Lab 3.0

S. No.	Ligands	Excretion Factors	
		CL_{plasma}	$T_{1/2}$
01.	Beta-sitosterol	13.205	0.541
02.	Charantin	3.879	1.191
03.	Embelin	2.391	1.178
04.	Eugenol	10.004	1.464
05.	Amp - B	1.96	3.982

Table no. 10: Toxicity analysis by using ADMET Lab 3.0

S. No.	Ligands	Toxicity Factors																			
		hERG blocker	hERG blocker (10um)	DILI	AMES mutagenicity	Rat oral Acute Toxicity	FDAMDD	Skin Sensitization	Carcinogenicity	Eye Corrosion	Eye Irritation	Respiratory	Human Hepatotoxicity	Drug-induced Nephrotoxicity	Cotoxicity	Hematotoxicity	Genotoxicity	RPMI-8226 immunotoxicity	A549 Cytotoxicity	Hek293 Cytotoxicity	Drug-induced Neurotoxicity
01.	Beta-sitosterol	0.092	0.659	0.28	0.468	0.346	0.288	0.93	0.582	0.976	0.993	0.822	0.31	0.138	0.138	0.136	0.113	0.053	0.161	0.275	0.563
02.	Charantin	0.134	0.269	0.764	0.623	0.035	0.077	1.0	0.333	0.001	0.123	0.316	0.577	0.548	0.932	0.47	0.002	0.095	0.907	0.683	0.014
03.	Embelin	0.095	0.266	0.446	0.159	0.454	0.368	0.949	0.273	0.971	0.999	0.96	0.551	0.204	0.187	0.357	0.002	0.047	0.114	0.113	0.036
04.	Eugenol	0.092	0.659	0.28	0.468	0.346	0.288	0.93	0.582	0.976	0.993	0.822	0.31	0.138	0.138	0.136	0.113	0.053	0.161	0.275	0.563
05.	Amp-B	0.001	0.0	0.0	0.534	0.561	0.0	0.112	1.0	0.002	0.0	0.042	0.002	0.611	0.0	1.0	0.174	0.031	0.634	0.846	0.036	0.0

Table no. 11: Toxicophore analysis by using ADMET Lab 3.0

S. No.	Ligand	Toxicophore Factors							
		Acute Toxicity Rule	Genotoxicity Carcinogenicity Rule	Non-Genotoxicity Carcinogenicity Rule	Skin Sensitization Rule	Aquatic Toxicity Rule	Non-Biodegradable Rule	SureChEMBRule	FAF-Drugs4Rule
01.	Beta-sitoste	0	0	0	0	1	0	0	0

	rol								
02.	Charantin	0	0	0	1	2	1	0	0
03.	Embelin	0	2	2	7	3	1	0	2 alerts
04.	Eugenol	0	0	0	5	0	0	0	2 alerts
05.	Amp-B	0	0	0	2	1	1	1	0