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IN-SILICO ANALYSIS OF GALLIC ACID DERIVATIVES FOR IDENTIFICATION OF NOVEL ALANINE RACEMASE INHIBITORS

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Keywords:

Alanine racemase, D-alanine, Gallic acid, Docking, ADMET, Antibacterial

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ABSTRACT: Background: In drug design development, Alanine racemase grabbed researchers' attention as it is a potential target for many antimicrobial agents due to its imperative role in bacterial cell wall synthesis. The irresponsible binge eating of antibiotics leads to drug resistance is a key issue of concern for the researchers. The development of multidrug resistance, responsible for causing immense human suffering, demands a renewed attempt to be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. This highlights the urgent need for a new and improved antibacterial agent with a new mode of action for use in clinical practice. **Method:** In the present work, gallic acid derivatives were designed and docked against the active site of the enzyme *Alanine racemase* (PDB code: 1SFT) using Schrodinger maestro module. Ligands with highest dock score were selected and subjected to ADMET analysis with the help of Qikprop module to study the pharmacokinetic profile of the drug candidate. **Result and Conclusion:** Based upon computational analysis, compounds (8, 11, 7, 9 and 6) were identified as potential ligands against the binding pockets of *Alanine racemase* and appeared to be the most promising leads for future drug discovery. ADMET calculation of these compounds falls within the bounds of the satisfactory range without any considerable violation of Lipinski's rule of five. This enables us to infer that these compounds appeared to be the most potential leads that can be investigated as new drug candidates with the improved therapeutic index for further drug design and development.

INTRODUCTION: *Alanine racemase* (EC 5.1.1.1) is an enzyme pervasive in prokaryotic cells and contributes as a therapeutic target for developing antibacterial agents due to its imperative role in bacterial cell wall synthesis. *Alanine racemase* belongs to the enzymes Isomerases is a pyridoxal-5'-phosphate (PLP) dependent homodimeric enzyme that catalyzes the reversible racemization of L- alanine and D-alanine.

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Alanine racemase is a key enzyme required for the synthesis of D-alanine, which acts as a pivotal precursor for the biosynthesis of peptidoglycan layers of bacterial cell wall [1]. Hence, its inhibition is found to be fatal to prokaryotes [2, 3]. D-cycloserine (DCS) is a broad-spectrum natural drug that interferes with peptidoglycan biosynthesis via the inactivation of the Alanine racemase enzyme. DCS is a structural analogue of D-alanine found to impede the enzyme's catalytic function by forming covalent bond with PLP cofactor.

Cycloserine and other substrate analogues due to lack of target specificity, results in cellular toxicity and other side effects, which prompted the researchers to explore and identify novel Alr inhibitors lacking substrate specificity with a different mechanism of enzyme inhibition [4].

The discovery of antimicrobial drugs conferred huge benefits on human health and changes the fate of mankind dramatically. Penicillin was the first antibiotic discovered by Alexander Fleming, which proven to be a boon in curing infectious diseases. As a result, antibiotics were regarded as wonder drugs and used generally for the management of infection caused by pathogens [5, 6]. However, a large number of people are reliant on antibiotics for maintenance and improvement of health. Antibiotics have become one of the most commonly prescribed drugs for curing various infections. This ultimately leads to the development of drug resistance that may often associate with irresponsible binge eating, which is a key issue of concern for the researchers. Now the greatest challenge of the twenty-first century is the development of multidrug resistance responsible for causing immense human suffering. The resistance problem demands that a renewed attempt be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics [7].

This highlights the need for a new and improved antibacterial agent that has not been compromised by bacterial resistance [8]. The reactive intermediates such as Reactive oxygen species (ROS) and oxygen radicals are generated as a byproduct of various metabolic reactions are the major contributor to many serious ailments such as asthma, cancer, cardiovascular disease and neurodegenerative disorder *etc*. Antioxidants combat free radical-mediated oxidative stress by neutralizing active species [9, 10]. The consumption of foodstuffs containing a good amount of antioxidants and antimicrobials plays an unbelievable role in maintaining human health. In recent years, therapeutically active plant-derived natural products and their derivative have gained attention due to their incredible antimicrobial and antioxidant efficacy and have emerged as a promising source for novel drug discovery for the prevention of many diseases. Some natural foodstuffs containing a decent amount of antioxidant and antimicrobial agents are summarized in **Table 1.**

TABLE 1: NATURAL ANTIOXIDANTS AND ANTIMICROBIALS FROM NATURAL SOURCES

S. no.	Source	Antioxidants/ Antimicrobials
1	Amla (*Emblica officinalis*)	1,8-Cineole [11]
2	Apple (*Malus domestica*)	Gallic acid, coumaric acid, chlorogenic acid, catechin, epicatechin, procyanidin, cyanidin-3-galactoside, quercetin-3-galactoside, quercetin-3-glucoside and quercetin-3-rhamnoside [12,13]
3	Buckwheat (*Fagopyrum esculentum)*	Rutin, quercetin, hyperin, and Catechins [14,15]
4	Carrot (*Daucus carota*)	Caffeic, chlorogenic, and p-coumaric acids [16]
5	Grapes (*Vitis vinifera*)	Malvidin, cyanidin, delphinidin, peonidin and petunidin, epigallocatechin, epigallocatechin-3-O-gallate, castalagin and prodelphinidin [17,18]
6	Guava (*Psidium guajava*)	Gallic Acid, Chlorogenic Acid, Ferulic Acid, Myricetin and Sinapic Acid [19]
7	Jamun (*Syzygium cumini*)	Quercetin [20]
8	Mango (*Mangifera indica L.)*	Gallic Acid, Chlorogenic Acid, Ferulic Acid, Myricetin and Sinapic Acid [19]
9	Pomegranate (*Punica granatum*)	Quercetin, kaempferol, luteolin, and Myricetin [21]
10	Soyabean (*Glycine max*)	Protocatechuic acid, p-hydroxybezoic acids and chlorogenic acid [22]

Gallic acid (GA, 3, 4, 5-trihydroxy benzoic acid) is a polyphenolic compound exclusively present in natural sources such as Oat flour (*Avena sativa*) [23], Apple (*Malus domestica*) [12, 13], Guava (*Psidium guajava*), Blacktea, Grapes (*Vitis vinifera*) [17, 18], Mango (*Mangifera indica L.)*, Brown alga (*Stypocaulons coparium)*, Berries, Walnut [19] and Mushroom species [24] *etc*. It is an important secondary plant metabolite that has been extensively studied for its antimicrobial potential such as antioxidant [25, 26], antibacterial, anticancer [27-29], antiviral [30], antifungal, cardioprotective [31], neuroprotective and anti-inflammatory activities *etc* [32]. Drug development is a time-consuming and cost-effective process in which the journey of a drug candidate commences from the pharmaceutical laboratory, undergoes rigorous testing and evaluation, then requires approval from regulatory authorities, and finally brings it to the market for the benefit of mankind [33].

There are thousands of drug candidates in the pipeline, many of which fell by the wayside, and only a few will make it to the market. With the advancement in computer-based drug design techniques, virtual screening of libraries of

chemical templates against modeled targets is done to predict their binding affinity. This has proven to be an effective method of scrutinizing the number of hits and streamlining the overall drug development process [34, 35]. In this present exploratory work, we have focused on the development of selective and novel natural *Alanine racemase* inhibitors-based antibacterial agents using structure based virtual screening. Maestro Glide module was utilized for performing *in-silico* studies. Ligands with high docking score were chosen and finally deduced by calculating their ADMET properties utilizing qikprop module.

MATERIAL AND METHODS: Docking calculations were consummated by using Maestro Schrodinger Glide (New York, USA) software. The ADMET parameters were deduced by using the qikprop tool. Laboratory for Preservation Technology and Enzyme Inhibition Studies, Department of Pharmaceutical Sciences, M.D. University, Rohtak, India was used for computational work.

MATERIAL AND METHODS:

Dataset Preparation: In the present work, molecular structures of gallic acid derivatives were drawn using ChemDraw Ultra software 12.0. The designed chemical compounds were subjected to *in-silico* evaluation with the help of Schrodinger maestro suite against the crystallographic structure of *Alanine racemase* (PDB ID: 1SFT) with 1.90 Å resolution in order to get better entities as a lead for future consideration.

Protein Preparation: A typical PDB structure is not befitting to be used directly for molecular modeling calculations because they ordinarily consist of many heavy atoms, metal ions, co-crystallize ligands, chain breaks and cofactors etc. The crystallographic structure of protein *alanine racemase* (PDB ID: 1SFT, with minimum resolution 1.90 Å) procured from *Geobacillus stearothermophilus* was selected and downloaded from Protein Data Bank. The protein structure loaded in the maestro workspace was bit unclear. In the preset option, protein was chosen to be viewed in cartoon representation by clicking pretty (in solvent) to clearly visualize the protein (grey), co-crystallized ligand (green) and water molecules (red spheres). The protein structure was prepared using protein preparation wizard Prepwiz [36-38]. During the protein preparation, extra groups and water molecules not participating in any kind of interaction were removed, missing atoms, side chains and loops were filled, bond orders were assigned, hydrogen atom were added and with the default settings, protein was preprocessed. To minimize steric clashes, optimization of hydrogen bonds was done. The energy-restrained structure of targeted protein was constructed by using OPLS3e force field. At last, minimization job was assigned to protein preparation wizard to get rid of all the anomalies and eventually prepared protein structure appeared in workspace **Fig. 1.**

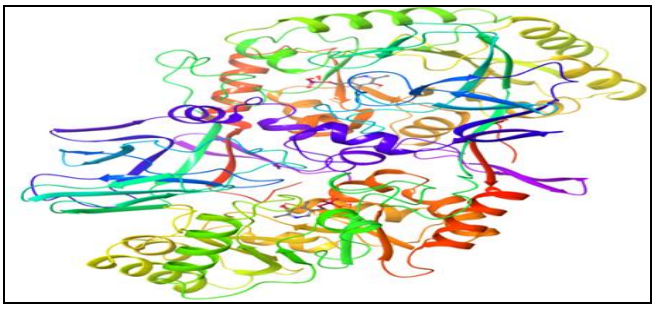


FIG. 1: PROTEIN STRUCTURE WITH PLP LIGAND (PDB CODE: 1SFT)

Ligand Preparation: The 2D structures of ligands were constructed using the Chemdraw ultra 12.0 and imported in MDL mol file format in maestro interface. For further conversion to more appropriate 3D conformations, energy minimization job was assigned to LigPrep tool of Maestro Molecular Modeling Interface of Schrodinger. Coordinates were fixed, ionization of structures done by Epik, tautomers were generated by submitting job to LigPrep tool. The partial charges were computed according to the OPLS3e force field at biological pH for energy minimization. Finally, energy minimized prepared ligand were appeared in the maestro navigator for molecular docking simulation [39].

Binding Site Prediction: Sitemap analysis was performed to pinpoint and select the most appropriate active pockets which showed the perfect binding site for ligands with the target enzyme. Around 5 binding regions were predicted and active site with largest volume, size, site score and D score was computed by using Site map application for docking calculations [40] shown in **Fig. 2.**

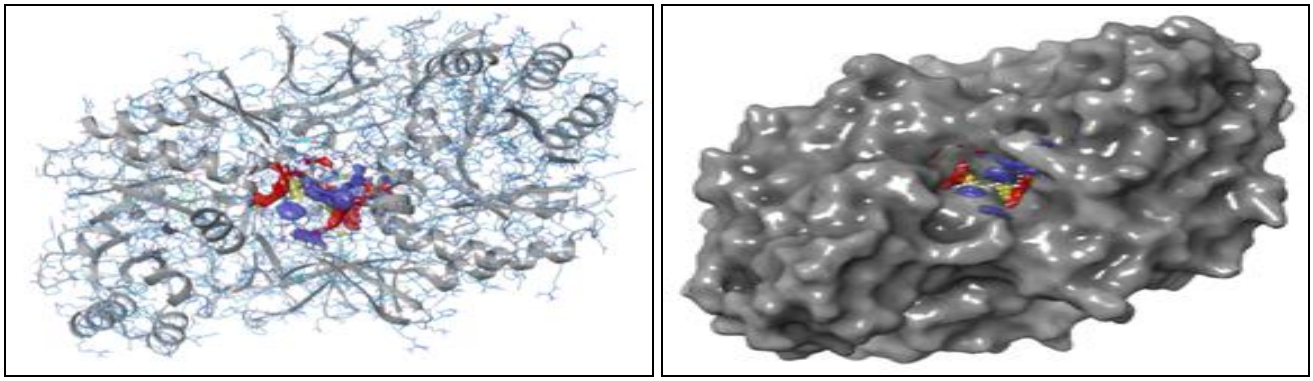


FIG. 2: ACTIVE/BINDING SITE OF ALANINE RACEMASE

Grid Generation: To perform docking calculations, the grid is generated using the maestro receptor grid generation module. Site map analysis revealed the binding site already occupied by the co-crystallized ligand, firstly, ligand atoms were selected in the workspace so that grid is generated around the binding site (appeared as purple box) **Fig. 3** and receptor grid generation job was assigned to create grid files. Grid files portray the active site of receptor for performing ligand docking job faster. Glide receptor grid generation was used to specify minimized protein grid using active site as calculated by site map and then the prepared protein was approached for docking studies [41].

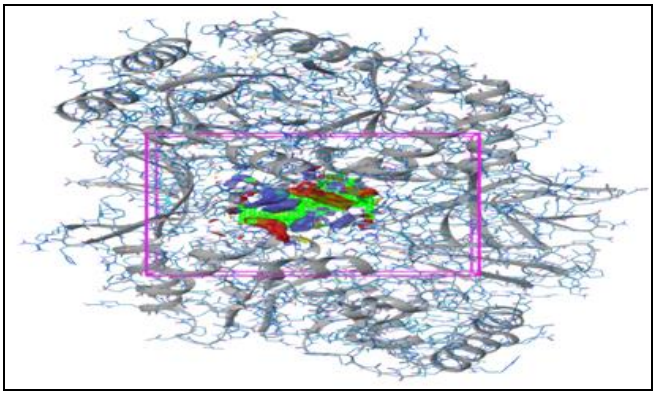


FIG. 3: GRID GENERATION IN BINDING SITE OF ALANINE RACEMASE (PDB CODE: 1SFT)

Molecular Docking: Molecular Docking is a computer-aided drug design approach in an era of modern drug discovery to predict drug-target interactions and docking pose depicting the most appropriate confirmation and orientation of the ligands with the binding pockets present on the enzyme surface were ranked *via* docking score function [40]. The Schrodinger, Inc. software platform Maestro was used for all computational calculations as it provides a wide platform for the virtual screening of chemical compounds against the target protein. Virtual screening is a direct and effective method to minimize the number of hits that interact with intended targets and then approached for experimental testing in wet laboratory aiming to achieve successful clinical trials for ensuring safe use in humans [37, 38, 42, 43].

ADMET Analysis: Qikprop module of Schrodinger's maestro molecular modeling package was utilized to generate ADMET (absorption, distribution, metabolism, excretion and toxicity) profile of designed molecules are given in **Table 4**. Nearly the set of eleven physicochemically significant descriptors and drug-likeness parameters were analyzed by Qikprop module to predict the pharmacokinetic profiles of the ligand. Descriptors analyzed by using this module were: QPlogS, QPlog HERG, QPlogBB, QPlogKp, QPPCaco, HBD, HBA, LogPo/w, %HOA and Lipinski's rule of five. Lipinski's rule of five was predicted for estimating drug like properties that would make it likely an orally active drug in humans using Qikprop module [44-47].

RESULTS AND DISCUSSION:

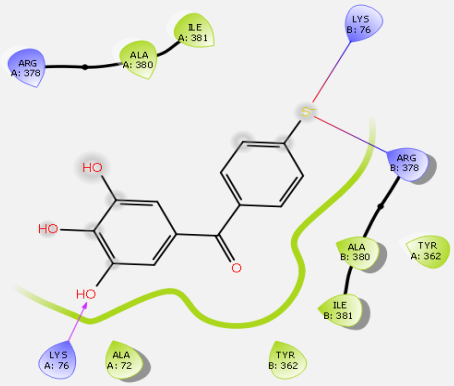
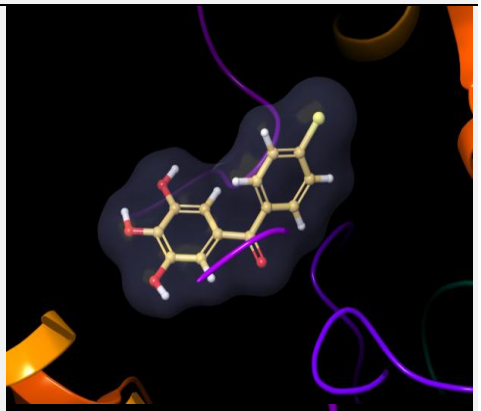
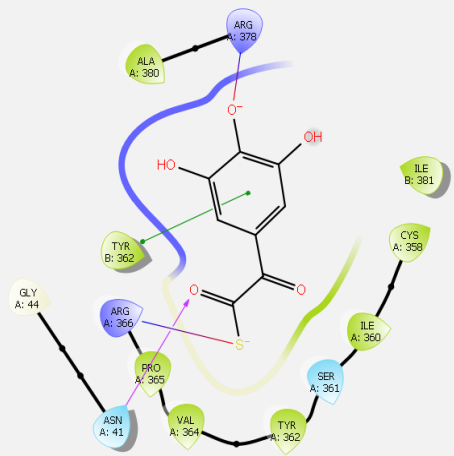
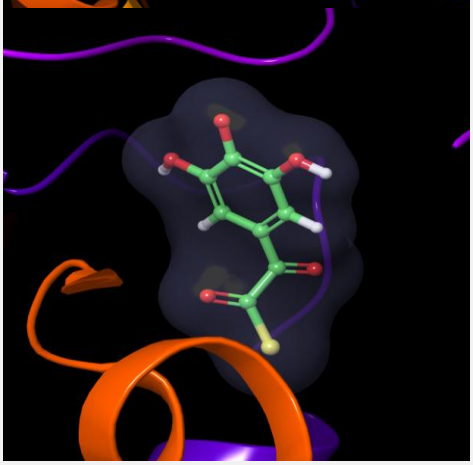
Molecular Docking: Newly designed ligands were docked against the catalytic site of *Alanine racemase* using Glide, Schrodinger Maestro Package of molecular docking software. Centre grid box was generated to locate all the active site residues for ligand binding. Ligand docking runs were performed with XP (extra precision) mode. At last, the ligands' interaction pattern, glide energy and docking score against *Alanine racemase* were obtained. Ligands with the best-docked pose or highest binding affinity (highest score with negative value) were selected for further studies, and the rest compounds with poor interaction were

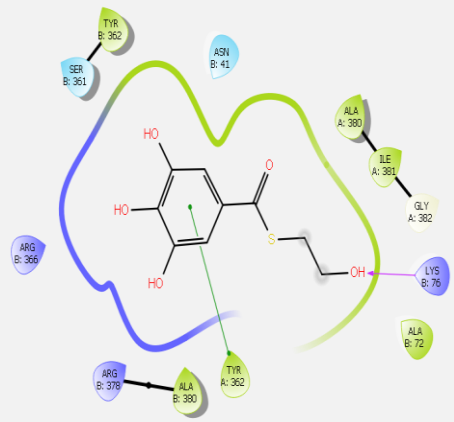
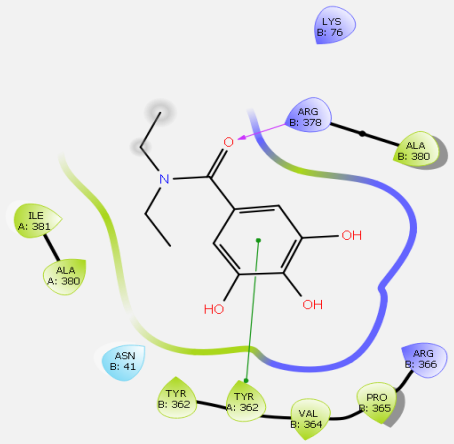
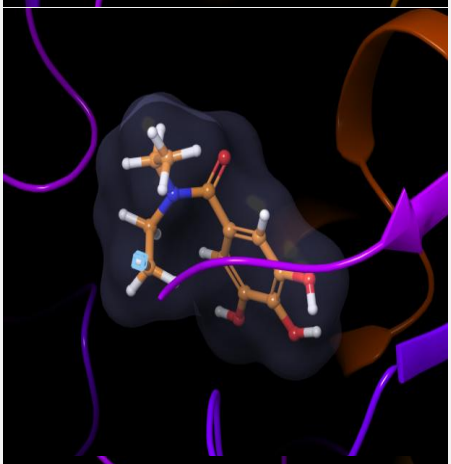
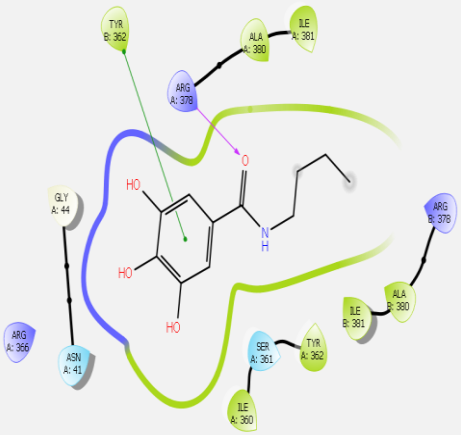
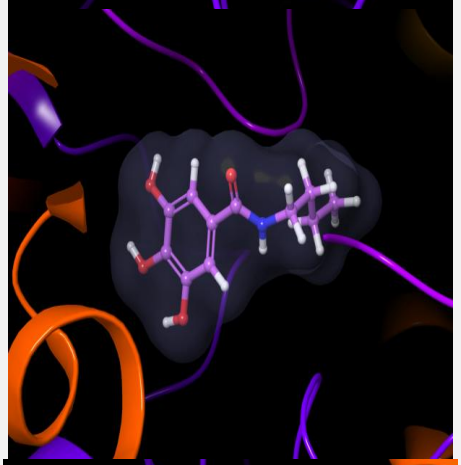
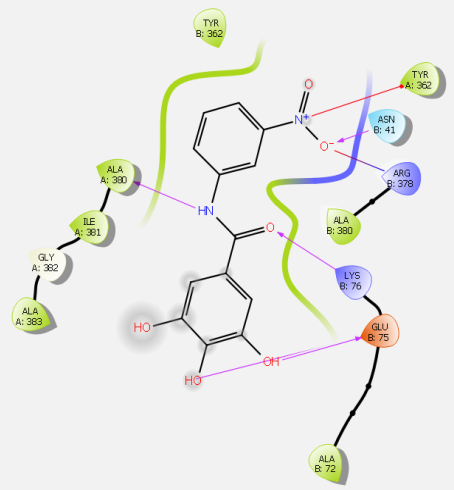
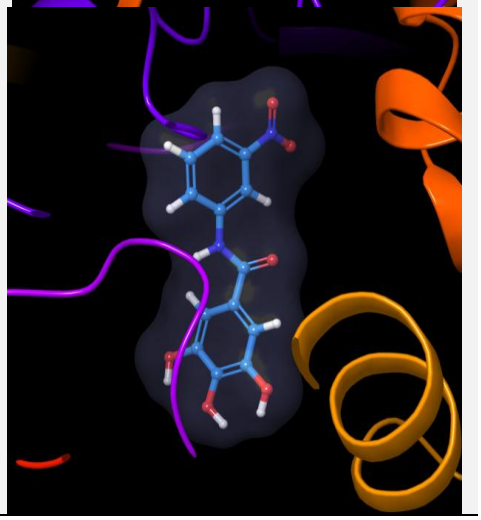
repudiated. The predicted binding pattern revealed that factors like H-bond formation, pi-pi stacking, hydrophobic interactions, bulkiness, salt bridges, positioning and alignment of groups are accountable for the ligand binding firmly with the active site of protein. The docking parameters of the selected ligands and standard drug are depicted in **Table 2.** Binding model of compounds 8, 11, 7, 9 and 6 exhibited good docking score (-7.379, -6.714, -6.203, -5.547 and -5.516, respectively) comparable to standard drug. The ligand-protein binding was visualized *via* interaction diagrams and docking poses depicted in **Table 3**. Binding mode of five most active compounds (8, 11, 7, 9, and 6) into *Alanine racemase* active site is shown in **Fig. 4.**

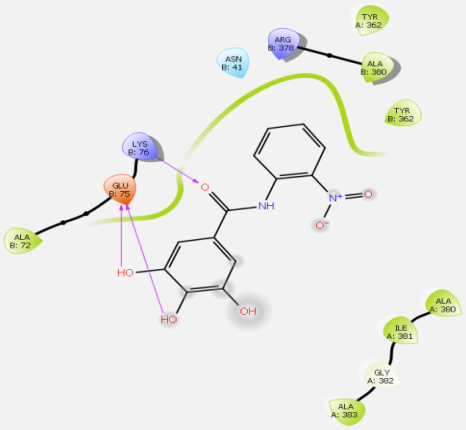
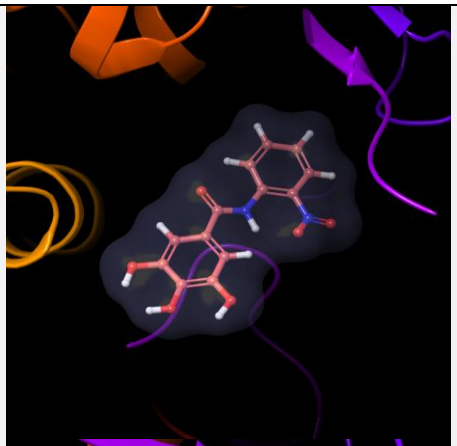
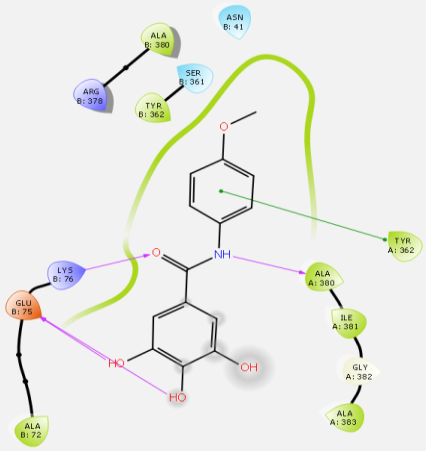
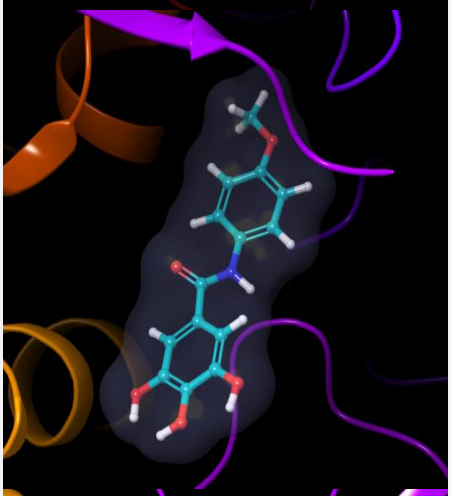
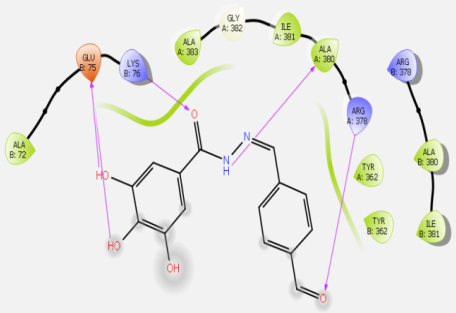
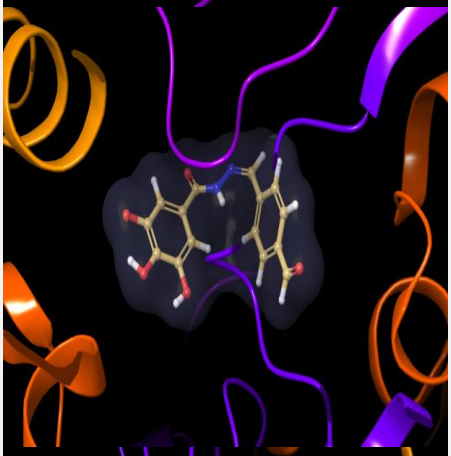
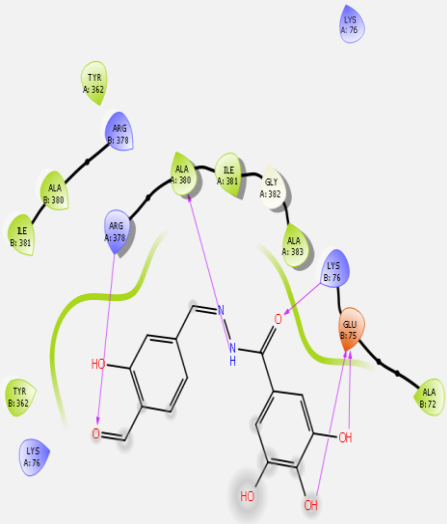
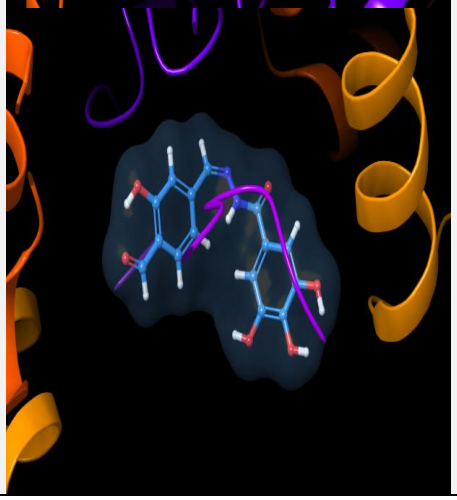
TABLE 2: DOCKING PARAMETERS OF DESIGNED COMPOUNDS

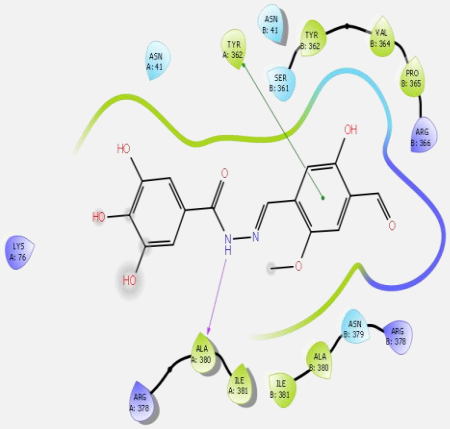
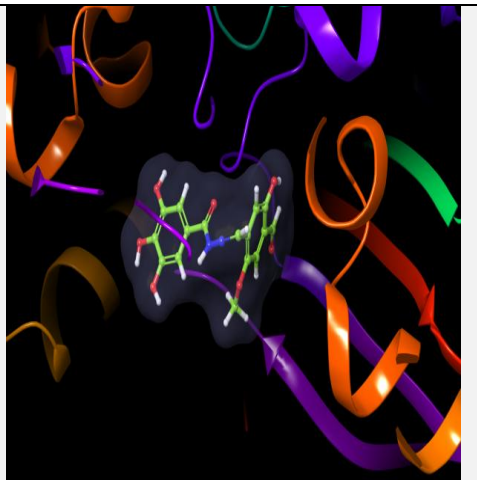
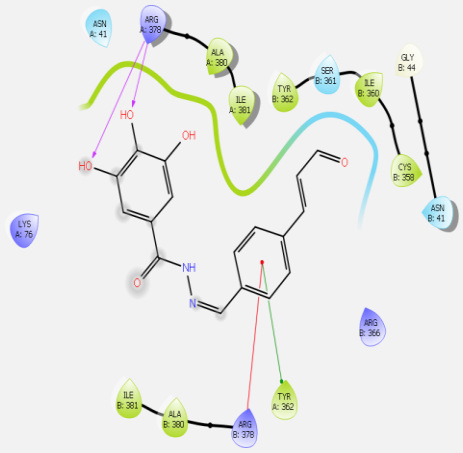
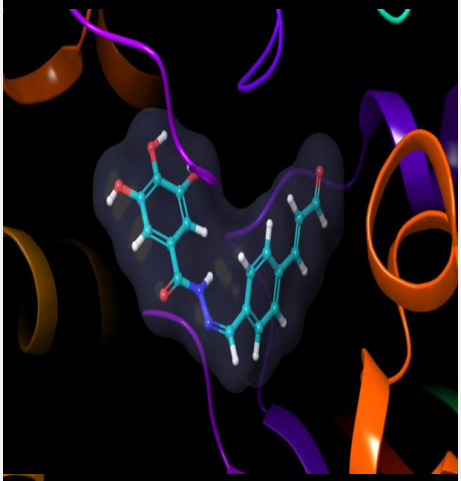
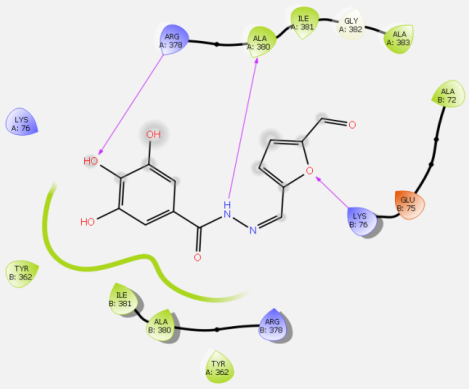
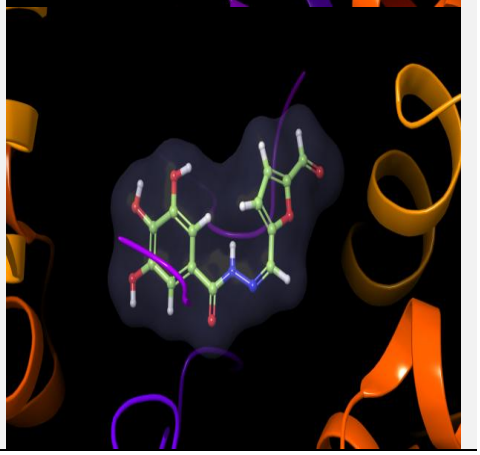
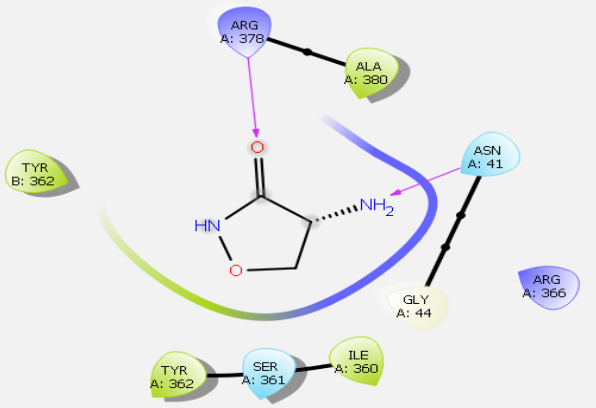
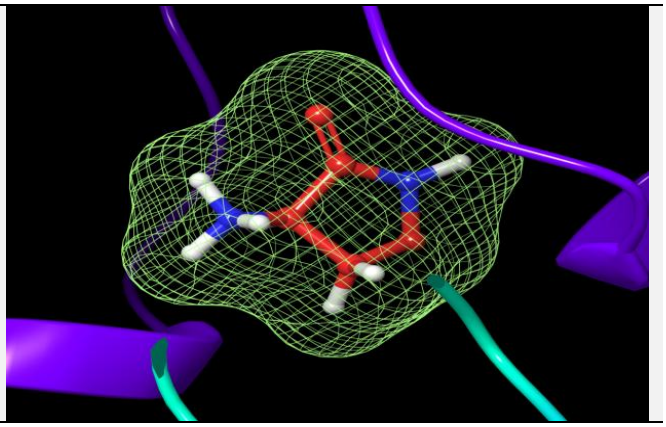
S. no.	Comp. Code	Docking Score	Glide energy	Glide gscore	Glide emodel
1	GA_1	-3.189	-22.516	-3.229	-30.724
2	GA_2	-4.495	-17.61	-4.704	-30.382
3	GA_3	-4.089	-34.024	-4.121	-46.14
4	GA_4	-4.458	-53.768	-4.493	-34.527
5	GA_5	-3.855	-38.059	-3.878	-34.831
6	GA_6	-5.516	-26.764	-5.535	-52.468
7	GA_7	-6.203	-38.351	-5.734	-53.995
8	GA_8	-7.379	-40.039	-6.223	-54.317
9	GA_9	-5.547	-51.695	-5.582	-57.364
10	GA_{10}	-5.416	-47.303	-5.718	-61.055
11	GA_{11}	-6.714	-45.719	-5.983	-57.258
12	GA_{12}	-4.523	-46.61	-4.558	-48.761
13	GA_{13}	-3.987	-33.839	-4.043	-48.162
14	DCS	-3.934	-20.009	-4.572	-25.571

TABLE 3: LIGAND PROTEIN INTERACTION DIAGRAMS AND DOCKING POSE OF GALLIC ACID DERIVATIVES

	Interaction Diagram	Interaction with amino acid Residues	Docking Pose
1		**Hydrophobic interactions:** ARG A:378, ALA A:380, ILE A:381, ARG B:378, ALA B:380, ILE B:381, ALA A:72, TYR B:362 **Hydrogen Bond:** LYS A:76. **Salt Bridge:** ARG B: 378, LYS B:76 **π- π stacking:** TYR A: 362	
2		**Hydrophobic interactions: :** ARG A: 366, PRO A: 365, VAL A: 364, TYR A:362, SER A:361, ILE A:360, CYS A:358, ALA A:380, ARG A: 378. **Hydrogen Bond:** ASN A:41 **Salt Bridge:** ARG A: 366, ARG A:378 **π- π stacking:** TYR B: 362	

3		**Hydrophobic interactions:** TYR B:362, SER B:361,ASN B:41, ILE A:381, GLY A:382, ALA A:380, ARG B:378, ALA B:380 **Hydrogen Bond:** LYS B:76 **π- π stacking:** TYR A: 362	
4		**Hydrophobic interactions:** ARG B: 366, PRO B: 365, VAL B: 364, TYR B:362, ILE A:381, ALA A:380, ASN B:41,ALA B:380, ARG B:378 **Hydrogen Bond:** ARG B:378 **π- π stacking:** TYR A: 362	
5		**Hydrophobic interactions:** ILE A:360, SER A:361, TYR A:362, ILE B:381, ALA B:380, ARG B: 378, ALA A:380, ILE A:381, ASN A:41,GLY A:44 **Hydrogen Bond:** ARG A:378 **π- π stacking:** TYR B: 362	
6		**Hydrophobic interactions:** ALA B:380, TYR A: 362, ARG B: 378, ALA A:380, ILE A:381, ASN B:41,ALA A:380, ILE A:381, GLY A:382, ALA A:381, ALA B:72 **Hydrogen Bond:** GLU B:75, LYS B:76,ASN B:41,ALA A:380 **Salt Bridge:** ARG B:378 **π- cation:** TYR A: 362	

7		**Hydrophobic interactions:** ALA B:380, TYR A: 362,ASN B:41, ALA A:380, ILE A:381, ASN B:41,ALA A:380, ILE A:381, GLY A:382, ALA A:383 **Hydrogen Bond:** GLU B:75, LYS B:76	
8		**Hydrophobic interactions:** SER B:361, TYR B: 362, ALA B:380, ILE A:381, ARG B:378, ALA A:380, ILE A:381, GLY A:382, ALA A:383 **Hydrogen Bond:** ALA A:380, GLU B:75, LYS B:76 **π- π stacking:** TYR A: 362	
9		**Hydrophobic interactions:** ALA A:383, GLY A:382,ILE A:381, ALA A:380, ARG A:378, TYR A:362,LYS B:76,GLU B:75, ALA B:72 **Hydrogen Bond:** ALA A:380, GLU B:75, LYS B:76, ARG A:378	
10		**Hydrophobic interactions:** ARG A:378, ALA A:380, ILE A:381, GLY A:382, ALA A:383, TYR B:362, LYS B:76,GLU B:75, ALA B:72 **Hydrogen Bond:** ALA A:380, ARG A:378, GLU B:75, LYS B:76	

11		**Hydrophobic interactions:** SER B:361, TYR B: 362, VAL B:364, PRO B:365, ARG B:366, ARG B:378, ALA A:380, ILE A:381, ALA B:380, ASN B:379, ARG B:378 **Hydrogen Bond:** ALA A:380 **π- π stacking:** TYR A: 362	
12		**Hydrophobic interactions:** ARG A:378, ALA A:380, ILE A:381, TYR B:362, SER B:361, ILE B: 360, CYS B:358 **Hydrogen Bond:** ARG A:378 **π- π stacking:** TYR A: 362 **Pi-cation:** ARG B:378	
13		**Hydrophobic interactions:** ALA A:380, GLY A:382,ILE A:381, ALA A:383, ARG A:378, TYR B:362, ILE B:381, ALA B:380, ALA B:378 **Hydrogen Bond:** ALA A:380, ARG A:378, LYS B:76	
DCS			

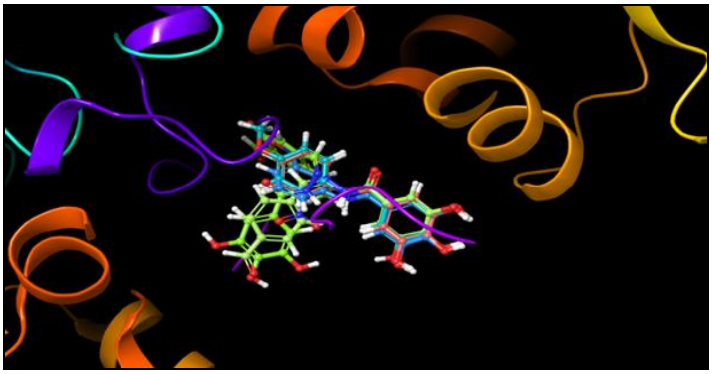


FIG. 4: BINDING MODE OF FIVE MOST ACTIVE COMPOUNDS (8, 11, 7, 9 AND 6) INTO *ALANINE RACEMASE* ACTIVE SITE

Interaction Studies of the Most Active Compounds: Comprehensive visualization of concerning the putative interplay between ligands and target protein unveil top five most active compounds on the basis of docking studies. The docking pose of the most active compound 8 revealed hydrophobic interactions among residues SER361, TYR362, ALA380, ILE381, ARG378, ALA380, ILE381, GLY382, ALA383. Three major hydrogen bonds were seen: one between hydroxyl group attached to the aromatic ring and residue GLU75, second between carbonyl group and LYS76 and third bond between amino group ALA380 residue along with pi-pi stacking with residue Tyr362.

The binding mode of second most active compound 11 displayed hydrophobic interactions among ligand and residues SER361, TYR362, VAL364, PRO365, ARG366, ARG378, ALA380, ILE381, ALA380, ASN379, ARG378. Hydrogen Bond formed between amino group and ALA380 residue and π- π stacking with phenyl ring of vanillin and residue TYR362. The close scanning of docking pose of the third most active compound 7 revealed hydrophobic interactions among ligand and channel of residues ALA380, TYR362, ASN41, ALA380, ILE381, ASN41, ALA380, ILE381, GLY382, ALA383. Hydrogen bond formation seen between carbonyl oxygen and GLU75, two hydroxyl groups attached to ring and LYS76. Examination of docking pose of fourth active compound 9 displayed that residues ALA383, GLY382, ILE381, ALA380, ARG378, TYR362, LYS76, GLU75, ALA72 were found to interact hydrophobically. Compound formed five hydrogen bonds: one with amino group and ALA380, residue GLU75 formed two H-bonds with two hydroxyl groups of phenyl ring, one between carbonyl oxygen and LYS B:76 and another one between carbonyl oxygen of aldehyde group and residue ARG378. Compound 6 displayed channel of residues ALA380, TYR362, ARG378, ALA380, ILE381, ASN41, ALA380, ILE381, GLY382, ALA381, ALA72 involved in hydrophobic interactions. Compound set up five hydrogen bonds: one between two hydroxyl groups of phenyl ring and residue GLU75, one betwixt carbonyl oxygen and LYS76, one between nitro group and ASN41 and another one between amino group and residue ALA 380. π- cation was seen between nitrogen atom of nitro group and residue TYR362. Salt bridge interaction exhibited by ARG378.

ADMET Prediction: ADMET prediction of drug candidates plays a significant role in generating lead compounds that hypothetically manifest acceptable ADMET performances during clinical trials. An ADMET prediction before clinical trials lowers the chances of failure and reduces the cost of drug development. ADMET profile of designed gallic acid derivatives predicted by using Qikprop module of Maestro suit shown in **Table 4.** Aqueous solubility (QPlogS), predicted IC_{50} value for blockage of HERG K^+ channels (QPlogHERG), apparent Caco-2 cell permeability estimation in nm/sec (QPPCaco), blood-brain barrier partition coefficient (QPlogBB), skin permeability estimation (QPlogKp), estimation of octanol-water coefficient (QPlogP o/w), hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), Percent human oral absorption (%HOA) and Lipinski's rule of five. The theoretical calculation of ADME parameters of ligands possessed in range values of QPlogS, QPlogHERG, QPlogBB, QPPCaco, QPlogKp, QPlogP o/w, HBD, HBA, %HOA within

the bounds of satisfactory range without any considerable violation of Lipinski's rule of five which made them ligands of choice for alanine racemase protein.

TABLE 4: QIKPROP SIMULATION STUDIES OF GALLIC ACID DERIVATIVES

Comp. Code	Mol. Wt.	QPlogPo/w	QPlogS	QPlog HERG	QPPCaco	QPlogBB	QPlog Kp	HBD	HBA	%HOA	Rule of Five
1	262.280	1.072	-2.544	-4.690	152.810	-1.305	-3.731	3	4.750	72.313	0
2	214.192	-0.729	-1.338	-3.607	51.734	-1.616	-5.101	4	6.250	53.349	0
3	230.235	-0.422	-1.437	-4.081	49.956	-2.007	-4.924	4	5.950	54.876	0
4	225.244	0.510	-1.805	-3.719	190.995	-1.340	-4.043	3	5.250	70.759	0
5	225.244	0.444	-2.113	-4.460	138.996	-1.684	-4.142	4	4.750	67.903	0
6	290.232	0.358	-2.760	-5.159	16.910	-2.538	-5.524	4	5.750	51.022	0
7	290.232	0.451	-2.682	-5.008	26.654	-2.272	-5.108	4	5.750	55.106	0
8	275.260	0.902	-2.731	-5.044	143.063	-1.589	-3.720	4	5.500	70.809	0
9	300.270	0.173	-2.585	-5.033	15.510	-2.681	-5.470	4	6.750	49.270	0
10	316.270	-0.026	-2.549	-4.870	6.343	-3.121	-6.245	2	6.500	41.454	0
11	346.296	0.236	-2.930	-4.959	7.003	-3.275	-6.214	4	7.250	43.456	0
12	326.308	0.664	-2.985	-5.390	13.011	-2.999	-5.375	4	6.750	50.780	0
13	290.232	-0.321	-1.930	-4.585	18.506	-2.464	-5.376	4	7.250	47.749	0
DCS	102.093	-1.947	0.687	-2.991	49.27	-0.41	-6.790	3	5.200	45.837	0

CONCLUSION: Based on computational analysis, compounds (8, 11, 7, 9 and 6) were identified as potential ligands against the binding pockets of *Alanine racemase* and appeared to be the most promising leads for future drug discovery. The upshot of this study provides cognizance of inhibitors derived from gallic acid with better dock scores and high binding affinity comparable to standard drugs. ADMET calculation of these compounds falls within the bounds of the satisfactory range without any considerable violation of Lipinski's rule of five. This enables us to infer that these compounds appeared to be the most potential leads that can be investigated as new drug candidates with the improved therapeutic index for further drug design and development.

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Human and Animal Rights: No Animals/Humans were used for studies based on this research.

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