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ADMET, Docking studies & binding energy calculations of some Novel ACE - inhibitors for the treatment of Diabetic Nephropathy

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Abstract

Diabetic Nephropathy (DN) is one of the major complications of diabetes mellitus, representing the leading of cause of chronic renal disease and a major cause of morbidity and mortality in both type 1 and type 2 diabetic patients. The Renin-Angiotensin-Aldosterone System (RAAS) has been implicated in the pathophysiology of DN, and suggests a therapeutic target for blocking this system. Therefore, inhibition of RAAS plays a crucial role in the treatment of DN and therapeutic intervention mostly involves administration of angiotensin converting enzyme (ACE) inhibitors and angiotensin AT_1 receptor blockers. In this current study, we have used computational methods to design 37 novel ACE-inhibitors and evaluated them for the interaction with the enzyme ACE through *insilico* analysis. The obtained results were compared with the standard drug enalapril to find out the potential inhibitors. Here we report that ligand 4 exhibited strongest inhibitory activity among all. All the analogs are also screened for their ADME & Toxicity profiles using *insilico* tools and ligand 9 is having better binding affinity next to ligand 4, and also having better ADMET profile when compared to that of ligand 4. Post docking calculations were also performed for the docked complexes in order to identify the individual ligand binding energies by employing Multi-Ligand Bimolecular Association with Energetics (Embrace).

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INTRODUCTION

Diabetes mellitus (DM) is a chronic disease affecting approximately 220 million people throughout the world. Uncontrolled DM often leads to several severe

complications including retinopathy, neuropathy and nephropathy [1].Of these, diabetic nephropathy (DN) is considered to be one of the major complications, characterized by persistent albuminuria, increased arterial blood pressure, and continuous decline in glomerular filtration rate (GFR)[2].Without specific treatment intervention, this condition eventually leads to end-stage renal disease (ESRD). Diabetic nephropathy is the most common cause of ESRD worldwide and affects approximately 30% of patients with type 1 DM and 20% of patients with type 2 DM [3]. Although there is no cure for DN, the rate of deterioration in renal function and therefore progression to ESRD can be delayed with treatment intervention. Several studies from past two decades provide evidence that controlling the levels of glucose in blood and reducing blood pressure are the key factors in the management of DN [4]. The renin-angiotensin system (RAS) has always been implicated in the regulatory functions of blood pressure and fluid homeostasis [5]. Hence blocking this RAS system is the first line therapy in the treatment of DN. Accordingly, Angiotensin converting enzyme (ACE) inhibitors and AT1 receptor blockers provide nephroprotective effect and delay the progression of DN[6]. In this current study, using computational methods we have designed 37 novel ACE-inhibitors and evaluated them for interaction with the enzyme ACE through *in silico* analysis.

MATERIALS AND METHODS

Selection & preparation of protein

Angiotensin converting enzyme (ACE) was retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/) with PDB Id- 1O8A with X-ray diffraction resolution of 2.00Å. ACE is responsible for conversion of Angiotensin - I to Angiotensin – II, which is responsible for increase in blood pressure, and Vascular Endothelium Dysfunction. Preparation of the retrieved protein was performed by using protein Preparation Wizard of Schrodinger suite 2010. Initially all the internal ligands, ions, metal elements, and water molecules were removed and hydrogens were added to satisfy the valances. Refinement of the loops was performed by using PRIME module, and hydrogen bonds were assigned. Energy minimization / geometrical optimization of the preprocessed protein structure were done by employing OPLS 2005 (Optimized Potentials for Liquid Simulations) with RMSD as 0.30.

Binding site characterization of the processed protein was performed by using SITEMAP 2.4 module[7] in which the hydrophilic (hydrogen bond acceptor & donor), hydrophobic, and metal binding regions were mapped which can be very useful in active site identification and also Structure based Drug designing (SBDD). Various regions of the active site of the retrieved protein can be seen in **Fig: 1**.

Selection of Lead moiety & Designing of ligands

The Lead, 2-(2-oxopropylamino)-4-phenylbutanoic acid is the common pharmacophore of the Carboxylic acid derivatives of Angiotensin Converting Enzyme Inhibitors. Carboxylic acid derivative of ACE inhibitor is selected as Lead moiety because of its optimum potency, higher bioavailability than phosphoric acid derivatives and low toxic profile than sulphonic acid derivative (captopril).

37 ligands were designed from the Lead compound by modifying the non pharmacophoric parts like R_1, R_2 and R_3. Modifications were primarily done at the non-pharmacophoric sites of the ACE inhibitors in order to maintain the original biological therapeutic activity. All the ligands were designed by using Accelrys – Symyx Draw 4.0. These ligands were designed according to the SAR properties of the carboxylic acid derivatives of ACE inhibitors. Structure of the lead scaffold and its sites of modification can be seen in **Fig: 2**. Newly designed 37 ligands were shown in **Table: 1.**

Preparation of ligands

Preparation of ligands was performed by using "LigPrep 2.4" module of Schrodinger Suite 2010. The simplest use of LigPrep is to produce a single, low-energy, 3D structure with correct chirality for each successfully processed input structure. LigPrep can also produce a number of structures from each input structure with various ionization states, tautomers, stereochemistries, and ring conformations, and eliminate molecules using various criteria including molecular weight or specified numbers and types of functional groups present [8].The ionization states in a given pH range of 7±2 (general pH of biological system) were generated by adding or removing protons from the ligand using EPIK 2.1 module. The option to account for metal binding is set by selecting Add metal binding states, can be used by Glide module when docking ligands to metalloproteins. OPLS 2005 Force Field was selected for energy minimization.

Molecular properties like Molecular weight, Hydrophobic component, Hydrophilic component, Total solvent-accessible volume, number of hydrogen bonds that would be donated, number of hydrogen bonds that would be accepted, partition coefficient of all the newly designed 37 ligands were studied by using "QikProp 3.3" module of Schrodinger Suite 2010 [9] and results were listed in **Table: 2**.

ADME & Toxicity Studies

Insilico ADME studies were performed by using ADME Descriptors algorithm of Accelrys Discovery studio 2.5 in which various pharmacokinetic parameters like Aq. Solubility [10], Human Intestinal Absorption [11],Plasma protein binding (PPB) [12],blood-brain-barrier (BBB) penetration [13], cytochrome P450 inhibition[14] and hepatotoxicity levels[15] were estimated for 37 ligands. Obtained results were cross checked with the standard levels listed in **Table: 3**

Toxicity profiling of all the 37 ligands were performed by employing Toxicity prediction – extensible protocol of Accelrys discovery studio 2.5. Toxicity profile includes screening for aerobic biodegradability, developmental toxicity potentials, AMES mutagenicity, carcinogenicity, and ocular & skin irritancy[16]. Teratogenicity effects of the ligands were studied by using an online tool, OSIRIS property explorer [17].

Receptor – Ligand Interactions (Docking Studies)

Receptor – Ligand interaction, generally docking studies were performed by using GLIDE 5.6 (Grid-Based Ligand Docking with Energetics) module in Extra Precision (XP) mode of Schrodinger Suite 2010[18,19].Glide docking algorithm consists of two main steps like receptor grid generation and ligand docking. In the first step, a three dimensional grid is generated by selecting a particular protein residue (from data obtained from SiteMap). Grid is constituted by receptor's shape and properties by sets of fields that provide relatively better accurate scoring of the ligand poses.

In another step, of molecular docking, Extra-precision (XP) docking, a different potential segregating procedure is employed to analyze the protein-ligand interactions. Then, the scoring is identified for the energy-minimized poses and the poses that pass the initial screens enter the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS-AA nonbonded ligand-receptor interaction energy. Then Emodel combines Glide Score, the nonbonded interaction energy, and the excess internal energy of the generated ligand conformation.

The docking score from Glide (GlideScore)[20] is entirely based on ChemScore.However, it also includes a steric-clash term, adds polar terms featured by Schrodinger to correct electrostatic mismatches, and has modifications to other terms:

GScore = 0.065 * Van der Waals energy + 0.130 * Coulomb energy + Lipophilic term (hydrophobic interactions) + H-bonding + Metal binding + BuryP

(Penalty for buried polar groups) + RotB (Penalty for freezing rotatable bonds)+ Site (Polar interactions in the active site).

Binding Energy Calculations

Post docking calculations like estimation of binding energies of the ligands with receptor were performed by employing the automated mechanism of Multi-Ligand Bimolecular Association with Energetics (MBAE)[21],using EMBRACE minimization of Macro Model 9.8 module of Schrodinger suite 2010. Embrace minimization was performed by opting energy difference mode. The calculation was performed first on the receptor, then on the ligand, and finally on the complex. The energy difference is then calculated using the equation:

$\Delta E = E_{complex} - E_{ligand} - E_{protein}$ (ΔE is the ligand binding energy)

RESULTS & DISCUSSION

ADME &Toxicity predictions

We have analyzed various phamrmacokinetic and pharmacodynamics properties of enalapril and its 37 newly designed analogs, among which were Aq. Pharmacokinetic properties were Solubility, Human Intestinal Absorption, Plasma protein binding (PPB), blood-brain-barrier (BBB) penetration, cytochrome P450 inhibition, and hepatotoxicity levels. Pharmacodynamics properties (toxicity profile) were Aerobic biodegradability, developmental toxicity potentials, AMES mutagenicity, carcinogenicity, ocular & skin irritancy, Teratogenicity effects. In this study, when the results were compared to the reference Level values and found that no single ligand is having BBB penetration, as ACE inhibitors should not cross BBB, to prevent the CNS adverse effects. ADME descriptor levels of the analogs that were obtained from the ADME Descriptors protocol of Accelrys Discovery studio were listed in **Table: 4.** Toxicity screening of the ligands along with enalapril was performed by using Toxicity prediction-extensible protocol and the results were tabulated in **Table: 5**. From these toxicity studies it was found that none of the ligands have shown mutagenicity and ligands like 31, 32 have shown carcinogenic characters in male rat models. As in general, ACE inhibitors were administered orally, skin & ocular irritancy characters can be neglected. Teratogenicity an effect of the ligands was studied by using an online tool has shown that none of the ligands were having reproductive effects. Few ligands have shown dose dependent toxicity characters.

Molecular Docking studies

To identify the molecular binding interactions of the analogs with the receptor, all the 37 ligands were docked into the active binding site of the enzyme ACE using Glide docking algorithm and the resulted XP GScore of the ligands were compared with enalapril (marketed potent ACE inhibitor). The docking result of the ligands and enalapril was listed in table: 6. The docking result revealed that the receptor-ligand complex was stabilized by hydrogen bonds, hydrophobic and electrostatic interactions. Among all the ligands, seven hydrogen bonds were formed between receptor active site residues and ligand 4 (**Fig: 3**) shown the highest dock score of -10.31 and enalapril (-6.9442) has six hydrogen bonds (**Fig: 4**). Most of the ligands have shown interactions with protein residues like GLN 281, HIS 383, GLU 384, LYS 511, ARG 522, TYR 523. About 14 ligands have shown better dock score than enalapril. Dock scores for all the 37 ligands and enalapril along with the interacted protein residues and bond distances were listed in **Table: 6**.

The aromatic hydroxyl group (-OH) in the pharmacophore of ligands 1, 4, 25, 26, 36, and 37 shown hydrogen bonding with protein residues like ASP 415 (ligand 1), GLU 411 (ligands 4, 25, 36, 37) and ASP 358. Common interaction sites in most of the ligands were carboxylic acid group which is a major pharmacophoric feature in carboxylic acid derivatives of ACE inhibitors that acts as Zinc binding site, terminal carboxylic acid attached to the

5 or 6 membered heterocyclics like piperdine and pyrrolidine, and their derived heterocyclic rings at R_1 position. The amine linkage ($-NH_2$) and carbonyl group (-C=O) of the ligands have shown binding interactions and plays a key role in the docking through their hydrophilic nature. Ligands showing better dock score than enalapril, have only 5 or 6 membered heterocyclic rings directly attached to carboxylic acid group ($-COO^-$) . Presence of the bulkier rings at R_1 decreases the binding affinity of the ligand towards the protein, which may be due to the steric hindrance of the methoxy and carboxylic groups attached to the bulkier rings.

In order to calculate the free energy of binding (FEB) of each ligand, Post docking calculations of the docked complexes were performed by using automated mechanism of Multi-Ligand Bimolecular Association with Energetics (MBAE). Total free energy of binding of each ligand is tabulated in **Table: 7**. The total free energy of binding is the difference energy of the complex and ligand & protein which includes solvation energy, Vander wall's energy, electrostatic energy, valence energy, and constraint energy.

Table 1: set of 37 newly designed ligands for ACE inhibitory activity

Ligand	R_3	R_2	R_1
Ligand 1	-OH	- CH_3	Pyrrolidine
Ligand 2	-OH	$-CH_3$	Piperidine 4 – carboxylic acid
Ligand 3	H	$-CH_3$	5,6-dimethoxy indoline
Ligand 4	-OH	-OH	Piperidine 4 – carboxylic acid
Ligand 5	H	H	Pyrrolidine-2-carboxylic acid
Ligand 6	H	$-CH_2OH$	Piperidine 2 – carboxylic acid
Ligand 7	H	$-C_2H_5$	Piperidine 2 – carboxylic acid
Ligand 8	H	$-C_2H_4NH_2$	Piperidine 2 – carboxylic acid
Ligand 9	H	$-CH_3$	Piperidine 2 – carboxylic acid
Ligand 10	H	$-CH_3$	1,4,5,6-tetrahydropyridine-2-carboxylic acid
Ligand 11	H	$-CH_3$	1,4-dihydropyridine-2-carboxylic acid
Ligand 12	H	$-CH_3$	1,2,5,6-tetrahydropyridine-2-carboxylic acid
Ligand 13	H	$-CH_3$	piperidine
Ligand 14	H	$-CH_3$	Piperidine 4 – carboxylic acid
Ligand 15	H	$-NH_2$	Pyrrolidine
Ligamd 16	H	$-NH_2$	Pyrrolidine-2-carboxylic acid
Ligand 17	H	$-NH_2$	Pyrrolidine-3-carboxylic acid
Ligand 18	H	$-NH_2$	indoline
Ligand 19	H	-OH	indoline
Ligand 20	H	-OH	Indoline-2-carboxylic acid
Ligand 21	H	-OH	Indoline-3-carboxylic acid
Ligand 22	H	$-NH_2$	Indoline-3-carboxylic acid
Ligand 23	H	-OH	2,5-dihydro-1H-pyrrole-2-carboxylic acid
Ligand 24	H	-OH	Pyrrolidine-2-carboxylic acid
Ligand 25	-OH	-OH	Pyrrolidine
Ligand 26	-OH	-OH	Pyrrolidine-2-carboxylic acid
Ligand 27	H	$-CH_3$	Pyrrolidine
Ligand 28	H	-OH	Pyrrolidine
Ligand 29	H	$-CH_3$	5,6-dimethoxy indoline-2carboxylic acid
Ligand 30	H	-OH	5,6-dimethoxy indoline-2carboxylic acid
Ligand 31	H	$-NH_2$	5,6-dimethoxy indoline-2carboxylic acid
Ligand 32	H	$-NH_2$	5,6-dimethoxy indoline
Ligand 33	H	-OH	5,6-dimethoxy indoline
Ligand 34	H	-OH	6,7-dimethoxy -1,2,3,4-tetrahydroisoquinoline
Ligand 35	H	-OH	1,2,3,4-tetrahydroisoquinoline
Ligand 36	-OH	$-NH_2$	Pyrrolidine-2-carboxylic acid
Ligand 37	-OH	$-CH_3$	Pyrrolidine-2-carboxylic acid

Table: 2: Molecular properties of the novel molecules obtained from Qikprop 3.3 module

Ligand	mol_MW	FOSA	FISA	volume	donorHB	accptHB	QPlogPo/w
1	320.388	294.207	185.721	1095.81	3	7.25	-0.726
2	376.452	341.731	212.884	1238.621	3	8.5	-0.122
3	380.397	219.279	309.353	1172.003	5	10.95	-2.229
4	380.397	219.279	309.353	1172.003	5	10.95	-2.229
5	334.371	222.175	222.387	1103.807	3	8.5	-0.762
6	378.424	230.52	232.073	1196.016	3	9.2	-0.581
7	376.452	310.352	173.944	1211.609	3	8.5	0.09
8	391.466	249.026	238.718	1228.431	5	9.5	-1.362
9	362.425	272.083	202.735	1188.604	3	8.5	-0.477
10	360.409	230.931	206.219	1176.759	3	8.5	0.202
11	358.393	181.209	180.089	1156.689	3	8.5	0.528
12	360.409	185.239	195.662	1164.836	3	8.5	-0.251
13	318.415	300.55	130.528	1105.419	2	6.5	0.207
14	362.425	243.026	219.421	1180.729	3	8.5	-0.565
15	305.376	227.796	152.176	988.532	4	7.5	-1.771
16	349.386	203.953	231.064	1091.939	5	9.5	-2.073
17	349.386	192.369	254.66	1102.636	5	9.5	-2.201
18	353.42	176.63	142.484	1142.1	4	7.5	-0.092
19	354.405	151.906	163.93	1155.091	3	8.2	0.275
20	398.415	124.982	237.075	1210.277	4	10.2	-0.274
21	398.415	125.176	244.864	1207.583	4	10.2	-0.351
22	397.43	121.236	210.009	1111.283	5	9.5	-0.991
23	348.355	115.518	247.612	1085.444	4	10.2	-1.739
24	350.371	201.318	249.003	1120.613	4	10.2	-1.65
25	322.36	256.474	213.059	1047.364	4	8.95	-1.943
26	366.37	211.988	294.808	1125.466	5	10.95	-2.378
27	304.388	295.754	114.59	1060.349	2	6.5	0.046
28	306.361	247.762	166.953	1043.327	3	8.2	-1.197
29	456.494	309.505	167.941	1361.722	3	10	1.227
30	458.467	240.367	245.349	1253.995	4	11.7	-0.661
31	457.482	253.501	216.084	1248.632	5	11	-0.885
32	413.472	288.126	155.836	1248.346	4	9	-0.164
33	414.457	293.779	150.852	1242.816	3	9.7	0.24
34	428.484	351.068	153.814	1347.167	3	9.7	0.167
35	368.432	166.828	162.101	1211.65	3	8.2	0.01
36	365.385	194.103	286.378	1101.779	6	10.25	-2.815
37	364.397	246.864	265.379	1167.773	4	9.25	-1.211

Table 3: standard levels of ADMET descriptors from Discovery studio 2.5

Aq. Solubility & Drug Likeness		BBB		CYP450		Hepatotoxicity		Int. Absorption	
level	intensity	level	intensity	level	value	level	value	level	value
0	Extremely low	0	Very high	0	Non inhibitor	0	Non toxic	0	Good
1	No, Very low	1	high	1	inhibitor	1	toxic	1	moderate
2	Yes, Low	2	medium	PPB				2	Poor
3	Yes, good	3	low	Level		% of binding		3	Very poor
4	Yes, optimal	4	Very low	0		<90%			
5	No, too soluble			1		>90%			
6	unknown			2		>95%			

Table 4: Predicted ADME profiles of the analogs

Ligand	BBB level	Human Intestinal Absorption level	Aq. Solubility level	Hepatotoxicity level	CPY2D6 level	PPB level
Ligand 1	3	0	3	0	0	0
Ligand 2	4	0	3	0	0	0
Ligand 3	3	0	3	1	0	2
Ligand 4	4	3	4	0	0	0
Ligand 5	3	0	4	0	0	1
Ligand 6	4	1	4	0	1	1
Ligand 7	4	0	3	0	1	1
Ligand 8	4	1	3	0	1	1
Ligand 9	4	0	3	0	1	1
Ligand 10	3	0	3	0	1	1
Ligand 11	3	0	3	1	0	2
Ligand 12	3	0	3	0	0	1
Ligand 13	3	0	3	0	1	1
Ligand 14	3	0	3	0	0	0
Ligand 15	3	0	3	0	0	1
Ligand 16	4	0	3	0	0	1
Ligand 17	4	1	3	0	0	1
Ligand 18	3	1	3	0	0	2
Ligand 19	3	0	3	0	0	2
Ligand 20	3	1	3	0	0	2
Ligand 21	4	1	3	0	0	2
Ligand 22	4	1	3	0	0	2
Ligand 23	4	1	4	0	0	1
Ligand 24	4	1	4	0	0	1
Ligand 25	4	0	4	0	0	0
Ligand 26	4	3	4	0	0	1
Ligand 27	3	0	3	0	0	1
Ligand 28	3	0	4	0	0	1
Ligand 29	4	1	3	1	1	1
Ligand 30	4	2	3	0	0	1
Ligand 31	4	3	2	1	0	1
Ligand 32	4	0	3	1	0	2
Ligand 33	4	0	3	0	0	2
Ligand 34	4	0	3	0	0	1
Ligand 35	3	0	3	0	0	1
Ligand 36	4	3	3	0	0	1
Ligand 37	4	1	3	0	0	1

BBB: Blood – Brain – Barrier, PPB: Plasma Protein Binding, CYP2D6: Cytochrome P_{450} enzyme inhibition using 2D chemical structure as input.

Table 5: Toxicity profile of 37 ligands using Toxicity Prediction – Extensible protocol of Accelrys Discovery Studio 2.5

Ligand	Aerobic Bio-Degradailability	AMES Mutagenicity	Developmental Toxicity Potential	Ocular Irritancy	Skin Irritancy	Skin Sensitizer	Carcinogenicity				
							Rodent	Female Mouse	Male Mouse	Female Rat	Male Rat
1	No	No	No	Yes	No	No	No	No	No	No	No
2	No	No	No	Yes	No	No	No	No	No	No	No
3	Yes	No	No	Yes	No	Yes	No	No	No	No	No
4	No	No	Yes	Yes	No	No	No	No	No	No	No
5	No	No	No	Yes	No	No	No	No	No	No	No
6	Yes	No	No	Yes	No	No	No	No	No	No	No
7	No	No	No	Yes	No	No	No	No	No	No	No
8	Yes	No	No	Yes	No	No	No	No	No	No	No
9	No	No	No	Yes	No	No	No	No	No	No	No
10	Yes	No	No	Yes	No	No	No	No	No	No	No
11	No	No	No	Yes	No	Yes	No	No	No	No	No
12	No	No	No	Yes	No	Yes	No	No	No	No	No
13	Yes	No	No	Yes	No	No	No	No	No	No	No
14	No	No	No	Yes	No	No	No	No	No	No	No
15	Yes	No	No	Yes	No	No	No	No	No	No	No
16	No	No	No	Yes	No	No	No	No	No	No	No
17	No	No	Yes	Yes	No	No	No	No	No	No	No
18	No	No	No	Yes	No	Yes	No	No	No	No	No
19	No	No	No	Yes	No	Yes	No	No	No	No	No
20	No	No	No	Yes	No	Yes	No	No	No	No	No
21	No	No	No	Yes	No	Yes	No	No	No	No	No
22	No	No	No	Yes	No	Yes	No	No	No	No	No
23	No	No	No	Yes	No	Yes	No	No	No	No	No
24	No	No	No	Yes	No	No	No	No	No	No	No
25	No	No	Yes	Yes	No	No	No	No	No	No	No
26	No	No	No	Yes	No	No	No	No	No	No	No
27	Yes	No	No	Yes	No	No	No	No	No	No	No
28	Yes	No	Yes	Yes	No	No	No	No	No	No	No
29	No	No	Yes	Yes	No	Yes	No	No	No	No	No
30	No	No	Yes	Yes	No	Yes	No	No	No	No	No
31	No	No	Yes	Yes	No	Yes	No	No	No	No	No
32	No	No	Yes	Yes	No	Yes	No	No	No	No	Yes
33	No	No	Yes	Yes	No	Yes	No	No	No	No	No
34	Yes	No	Yes	Yes	No	Yes	No	No	No	No	Yes
35	No	No	No	Yes	No	Yes	No	No	No	No	No
36	No	No	No	Yes	No	No	No	No	No	No	No
37	No	No	No	Yes	No	No	No	No	No	No	No

Table 6: Docking results and Protein-ligand binding interactions of 37 novel ligands & enalapril with the target protein Angiotensin converting enzyme (PDB ID: 1O8A)

Ligand name	XP GScore	No of H bonds	Amino acids	H bond Dist. (Å)
d 4	-10.3082	7	ARG 124	1.573
			ASN 85	2.086
			ASN 70	1.800
			GLU 411	2.148
			TYR 523	2.139
			GLU 143	1.479 2.021
d 9	-9.26875	5	HIS 353	2.195
			LYS 511	1.757 1.901
			GLN 281	1.915 2.116
d 16	-8.82356	6	GLU 143	2.224 1.716
			ASN 70	2.388 1.887
			LYS 368	2.322
			ASN 66	1.814
d 24	-8.50432	6	HIS 383	2.220
			GLU 384	1.712
			HIS 387	1.886
			ALA 356	1.644
			ARG 522	1.896
			TYR 523	1.773
d 36	-8.46714	2	GLU 411	2.017
			ASN 66	2.014
d 2	-8.32913	2	ASN 70	2.153
			ASN 85	2.055
d 26	-8.06435	5	ARG 522	1.886
			ASP 358	1.943
			GLU 384	1.996
			HIS 383	1.759

			TYR 523	1.893
d 6	-7.70781	3	GLN 281	2.199 2.489
			LYS 511	1.824
d 11	-7.52812	4	GLN 281	1.861 1.892
			ASN 277	2.189
			LYS 511	1.886
d 12	-7.42674	5	ARG 522	1.785
			GLU 384	1.682
			HIS 383	2.294
			TYR 523	1.595
			ALA 356	2.087
d5	-7.18166	5	ARG 522	2.095
			TYR 523	1.725
			HIS 383	2.192
			GLU 384	2.023
			ALA 356	2.474
d 23	-7.16059	3	LYS 511	2.153 2.257
			GLN 281	1.866
d 14	-7.05793	4	ASN 66	2.059
			GLU 384	1.772
			HIS 383	1.876
			TYR 523	2.110
d 1	-6.98373	4	ASP 415	1.643
			LYS 511	2.031
			GLN 281	2.024
			ALA 354	2.033
ENALAPRIL	-6.94422	6	ALA 356	2.392 2.202
			GLU 384	1.824
			HIS 383	2.383
			TYR 523	2.030
			ARG 522	2.011
d 31	-6.93853	2	ARG 522	1.844
			ALA 356	2.106
d 27	-6.86177	4	ASN 70	1.925 2.324
			GLU 143	2.208
			LYS 368	2.397
Ligand 3	-6.63594	2	ARG 522	1.894
			TYR 523	1.972
d 17	-6.52496	4	ASN 70	2.097 2.107
			ASN 66	2.217
			GLU 143	1.599
d 32	-6.46851	2	ASN 66	1.960
			ASN 70	1.964
d 7	-6.40649	3	ARG 522	1.819
			TYR 523	1.702
			GLU 384	1.906
d 29	-6.29475	3	TYR 360	2.098
			ALA 356	1.955
			GLU 384	2.068
d 15	-5.9104	3	GLU 162	2.257
			HIS 353	2.048
			LYS 511	1.763
d 8	-5.64248	3	GLU 162	2.062
			ALA 354	2.061
			TYR 523	2.492
d 37	-5.40506	2	ASN 66	1.857
			GLU 411	1.904
d 18	-5.39369	2	ALA 356	1.946 2.147
d 22	-5.37216	5	ALA 356	2.482 2.062
			GLU 384	2.425
			HIS 383	1.976
			TYR 523	1.996
d 10	-5.25295	3	ALA 356	2.052 2.121
			GLU 384	1.733
d 13	-4.88648	3	HIS 353	2.401
			ALA 354	2.023
			LYS 511	2.056
d 25	-2.91444	4	GLU 411	2.435
			TYR 523	2.188
			LYS 368	2.110
			ASN 70	1.819
d 34	-2.45262	2	HIS 383	1.766
			ALA 354	1.877
d 21	-1.77973	3	ALA 356	2.271

			HIS 513	2.230
			LYS 511	1.656
d 28	-1.55394	4	GLN 281	1.998 2.206
			HIS 353	2.097
			LYS 511	1.985
d 35	-0.1516	4	ALA 356	1.903
			GLU 384	1.719
			HIS 383	1.914
			TYR 523	1.977
d 33	0.062574	2	GLN 281	1.981
			LYS 511	1.805
d 30	0.316816	3	ALA 356	1.964 2.239
			GLU 384	2.119
d 19	1.38718	4	HIS 353	2.303
			GLN 281	1.998 2.430
			LYS 511	1.809
d 20	2.857868	4	GLN 281	2.107 2.108
			ASN 277	2.050
			LYS 511	2.000

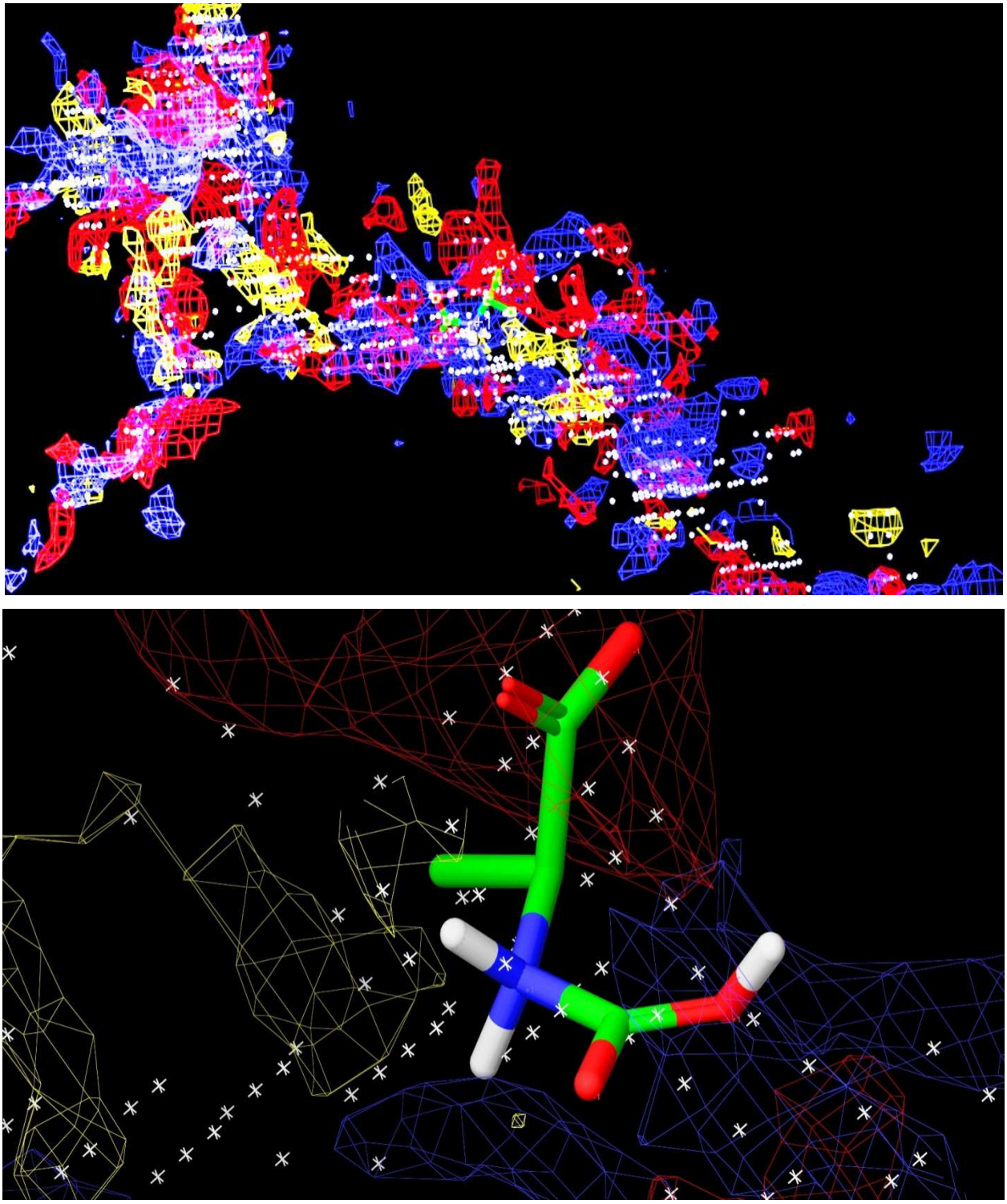
ARG: Argenine, LYS: Lysine, ALA: Alanine, ASN: Asparagine, HIS: Histidine, GLN: Glutamine, GLU: Glutamic acid, TYR: Tyrosine, THR: Threonine.

Table 7: Binding energies of 37 novel analogs & enalapril docked protein-ligand complexes

Ligand	MBAE Del Total Energy (ΔE)	MBAE Complex Total Energy ($E_{complex}$)	MBAE Rec Total Energy ($E_{protein}$)	MBAE Lig Total Energy (E_{ligand})
Ligand 4	-815.734882	-9843.286484	-8436.27145	-591.280151
Ligand 9	-638.954376	-9687.354366	-8436.27145	-612.12854
Ligand 16	-533.713848	-10141.53388	-8436.27145	-1171.548584
ligand 24	-533.856907	-9607.26416	-8436.27145	-637.135803
Ligand 36	-719.442474	-9791.896053	-8436.27145	-636.182129
Ligand 2	-798.068043	-9720.137741	-8436.27145	-485.798248
ligand 26	-636.012642	-9721.255772	-8436.27145	-648.97168
Ligand 6	-657.840881	-9655.585354	-8436.27145	-561.473022
Ligand 11	-463.491543	-9512.426445	-8436.27145	-612.663452
Ligand 12	-482.254459	-9545.840363	-8436.27145	-627.314453
Ligand 5	-558.921848	-9611.7005	-8436.27145	-616.507202
Ligand 23	-730.761753	-9736.037781	-8436.27145	-569.004578
Ligand 14	-509.315144	-9418.780533	-8436.27145	-473.193939
Ligand 1	-479.384377	-9302.728519	-8436.27145	-387.072693
ENALAPRIL	-517.243534	-9543.426239	-8436.27145	-589.911255
Ligand 31	-456.10804	-9943.196018	-8436.27145	-1050.816528
Ligand 27	-511.648926	-9270.433681	-8436.27145	-322.513306
Ligand 3	-687.061367	-9620.427696	-8436.27145	-497.094879
Ligand 17	-439.667042	-10016.47316	-8436.27145	-1140.534668
Ligand 32	-462.021957	-9064.724941	-8436.27145	-166.431534
Ligand 7	-535.002762	-9442.076244	-8436.27145	-470.802032
Ligand 29	-550.080853	-9473.164833	-8436.27145	-486.812531
Ligand 15	-462.984894	-9324.112301	-8436.27145	-424.855957
Ligand 8	-687.061367	-9620.427696	-8436.27145	-497.094879
ligand 37	-523.136749	-9577.721004	-8436.27145	-618.312805
Ligand 18	-186.065338	-9197.151669	-8436.27145	-574.81488
Ligand 22	-418.210197	-9808.612324	-8436.27145	-954.130676
Ligand 10	-534.434776	-9465.23531	-8436.27145	-494.529083
Ligand 13	-411.619144	-9242.15097	-8436.27145	-394.260376
ligand 25	-533.600948	-9279.19622	-8436.27145	-309.323822
Ligand 34	-487.100201	-9228.770515	-8436.27145	-305.398865
ligand 21	-1006.483616	-9477.045097	-8436.27145	-34.290031
Ligand 28	-639.069996	-9363.563919	-8436.27145	-288.222473
Ligand 35	-434.586662	-9232.798878	-8436.27145	-361.940765
Ligand 33	-547.617397	-9190.446098	-8436.27145	-206.557251
Ligand 30	-858.041714	-9314.002361	-8436.27145	-19.689198
Ligand 19	-566.571293	-9238.615036	-8436.27145	-235.772293
Ligand 20	-872.65192	-9381.763924	-8436.27145	-72.840553

Fig 1: binding site characterization of the protein Angiotensin converting enzyme (1O8A)

(a) Positions of various binding interaction features of protein 1O8A



(b) Fig showing the interactions of the internal ligand of 1O8A protein.

In the above figs, Contour maps (site maps) are generated, producing hydrophobic (yellow mesh) and hydrophilic maps. The hydrophilic maps are further divided into donor (blue mesh), acceptor (red mesh), and metal-binding regions (pink mesh).

Fig 2: Structure of the lead scaffold and its sites of modification

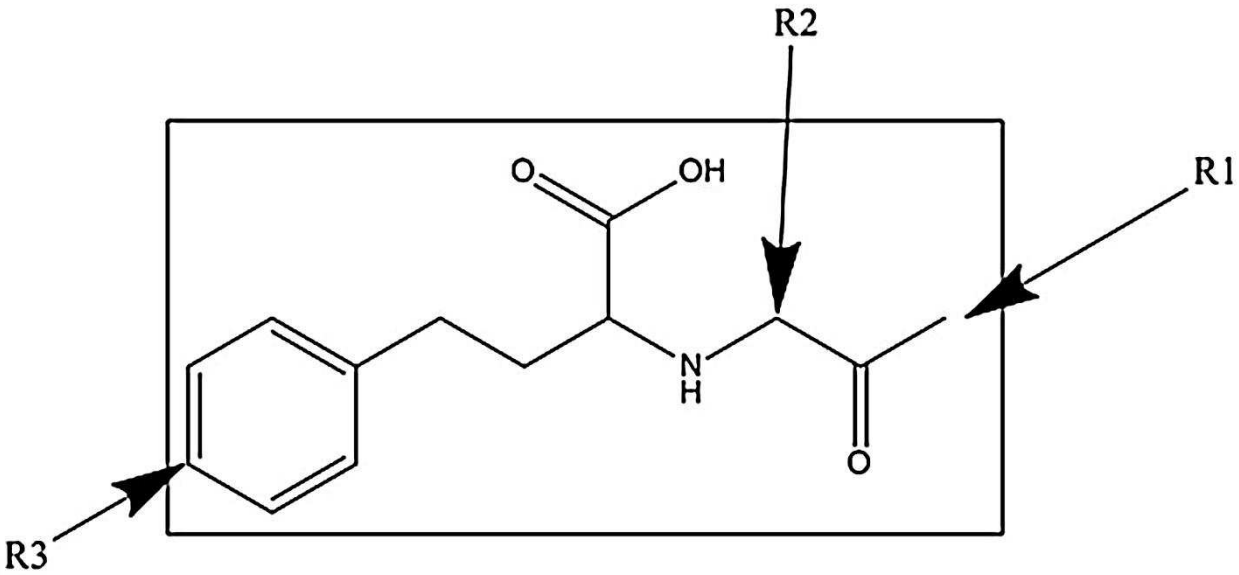
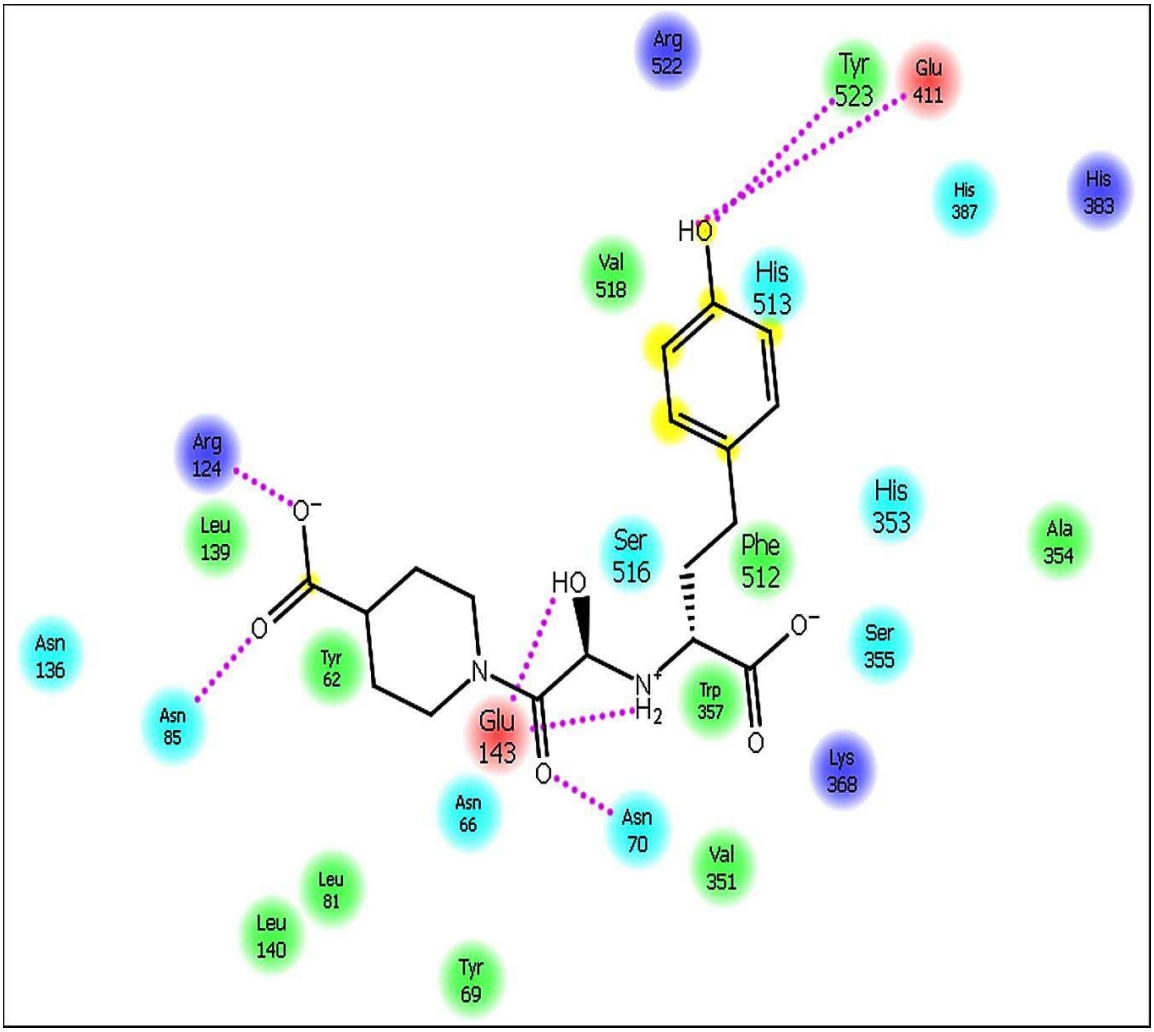
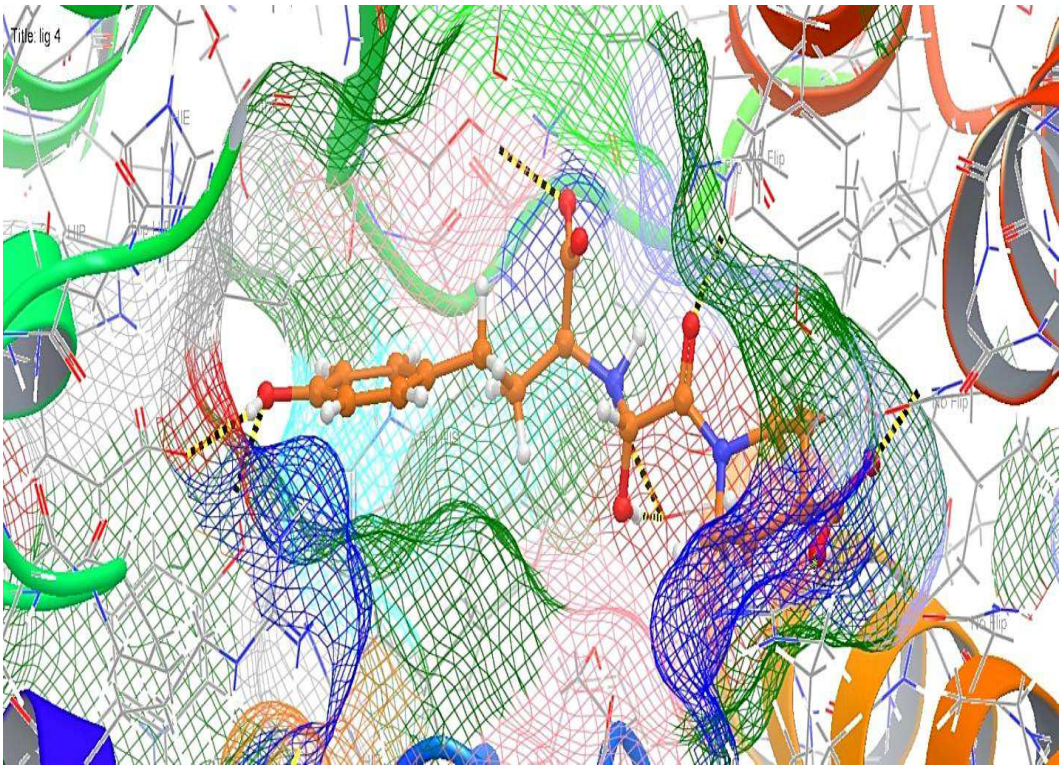


Fig 3: binding orientations of ligand 4 with Angiotensin Converting Enzyme (PDB ID: 1O8A)

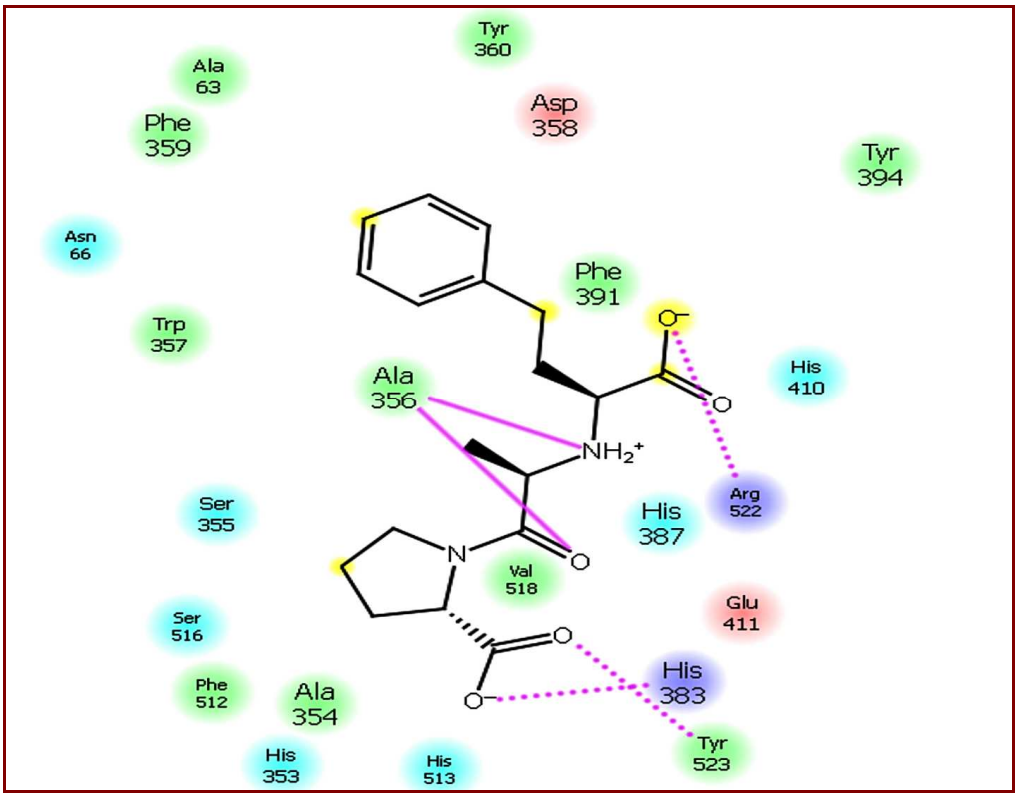


a) Ligand 4 is showing 7 hydrogen bonds (pink colour dotted lines) with the protein residues. Arg-argenine, Asn – Aspertamine, Glu – glutamine, Tyr – tyrosine

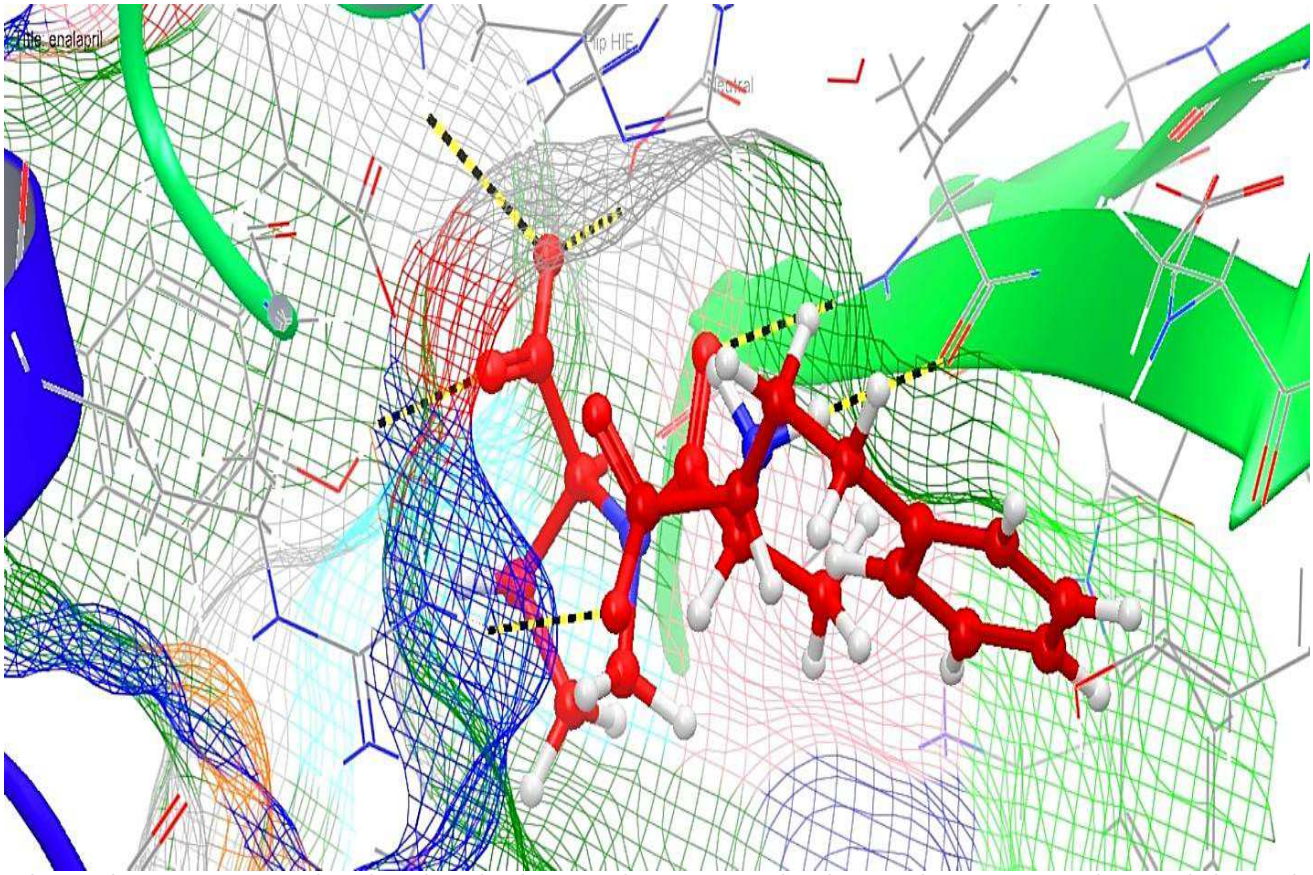


(b) docking orientation of ligand 4 (orange colour) in the active binding site of protein. Mesh represents the active site pocket of the protein ACE. Yellow dotted lines indicate the hydrogen bonds formed between ligand 4 and protein.

Fig 4: Docking orientation of enalapril at the active site if protein ACE(1O8A)



(a) Ligand 4 is showing 5 hydrogen bonds (pink colour dotted lines) with the protein residues. Arg-argenine, Ala - alanine, His - histidine, Tyr – tyrosine.



(b) Binding interaction of enalapril (red colour) at the active site binding pocket represented in mesh form of ACE.

CONCLUSION

In this study we have designed a set of 37 novel molecules and performed docking simulations in order to identify their binding affinity and binding energy towards the protein angiotensin converting enzyme and tested for their ADME & Toxicity profiles using *Insilico* tools. Among all the 37 molecules and enalapril, (marketed drug) ligand 4 has shown highest dock score (XP GScore) .Ligand 9 has shown the best dock score next to ligand 4 with better ADMET profiles. Binding energies in the protein – ligand interactions explain how fit the ligand binds with target protein.

Examination of the binding interactions of the ligands helps in elucidating the reasonable and appropriate structural features of ligand which increase the binding affinity and therapeutic efficacy. Presence of the bulkier ring structures at R_1 position might decrease the overall fitness of the ligand and presence of the aromatic hydroxyl group at R_3 has shown better binding fitness by forming an extra hydrogen bond.

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